



AGILENT BOND ELUT CERTIFY AND CERTIFY II METHODS MANUAL

The Measure of Confidence



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The following are analytical methods provided to help in analyte selection, design and development, and subsequent validation by the laboratory.

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Dear Valued Customer,

The task of identifying and quantifying drugs of abuse and pharmaceuticals in a wide range of sample types is one of the biggest challenges facing forensic toxicology laboratories. The variables seem insurmountable from the unpredictable sample consistency and makeup to an ever-changing list of controlled substances, designer drugs, and other contaminants. These analytical challenges require a range of solutions to ensure your laboratory reaches the correct answer without fail.

Agilent comprehensive analytical solutions help you to deliver on your promises


Agilent solutions include sample preparation, instruments, analyzers, columns, and supplies that work together to achieve the highest levels of integrity for your lab. We want to make OUR knowledge YOUR knowledge. This in-depth manual is a great step to assist in advancing your science and achieving your goals with a partner you can trust.

**Agilent Bond Elut Certify Solid Phase Extraction (SPE):
Your first step in forensic toxicology analysis**

This manual features sample preparation methods using Agilent's Bond Elut Certify and Certify II SPE products, focusing on extraction protocols covering controlled substances, drugs of abuse, and pharmaceuticals in a range of sample types, including urine, blood, meconium, and plasma. Reliable, consistent, and effective, **Bond Elut Certify** has been the SPE product of choice for forensic scientists for decades. Quickly and successfully develop and implement an SPE method for liquid or gas chromatography. We offer a complete range of sample preparation solutions.

- **Agilent Bond Elut solid phase extraction (SPE)**, comprising over 40 different polymeric and silica-based functionalities, available in a variety of cartridge and plate formats.
- **Agilent Bond Elut QuEChERS**, for easy and reproducible salt extraction and dispersive-SPE cleanup of compounds from complex matrices.
- **Agilent Chem Elut solid supported liquid extraction (SLE)**, for easy and reproducible cleanup using the same principles as liquid-liquid extractions, without the complications.
- **Agilent Captiva filtration**, for mechanical and chemical filtration in a variety of formats to simplify sample preparation methods and deliver the best sample hygiene.
- **Agilent vacuum manifolds and accessories**, to support cartridge and 96-well plate extraction workflows for maximum efficiency.

Our team of scientists continues to support your analytical needs, so be sure to check our sample prep pages at agilent.com/chem/sampleprep for new applications and product developments.



April DeAtley
Sample Preparation Product Manager

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

Summary of Bond Elut Certify and Certify II Mixed Mode Extraction

Section 1 – GENERAL INFORMATION

In testing for drugs of abuse in biological fluids, Agilent Bond Elut Certify mixed mode sorbent takes advantage of nonpolar, polar, and ion-exchange properties to ensure rapid, reproducible, simple, and clean extraction of many drug classes. Because Bond Elut Certify exhibits a variety of sorbent-analyte interactions, it can be used either as a general clean-up approach across a range of drug classes, or employed for specific extractions for instrumental confirmation of drugs and metabolites.

The multi-faceted performance of Bond Elut Certify arises from its mixed mode composition. The use of a bonded phase containing a medium-length hydrocarbon chain allows for some exposure of the polar silica surface. This optimizes polar and nonpolar interactions of the drugs and matrix interferences with the sorbent. The second bonded phase, a strong cation-exchanger, is optimized for capacity. Too many ion-exchange sites result in high background and difficult elution, whereas too few produce low recoveries. The complex sorbent is specially tested, first through rigorous ion-exchange and nonpolar chromatographic checks of the component phases, then through a final recovery using the completed product. Because the three modes of retention – polar, nonpolar, ion-exchange – are precisely matched, Bond Elut Certify is ideal for general drug analysis or the extraction of specific basic, acidic, or neutral drugs. What follows is a general discussion of how each drug class interacts with the Bond Elut Certify sorbent to allow for its extraction from complex biological matrices.

Section 2 – INITIAL CONSIDERATIONS

The Bond Elut Certify cartridge is conditioned first with methanol to open up the coiled hydrophobic portion of the sorbent and activate it toward interaction with a polar matrix. Further conditioning with buffer removes excess methanol and places the sorbent bed in an environment as similar to the matrix as possible. This allows for maximum sorbent-matrix

interaction and reproducible recoveries. The extraction of drugs from a complex biological matrix such as plasma or urine requires that pH, ionic strength, and viscosity be controlled. This is accomplished by dilution of the sample with buffer.

Section 3 – BASIC DRUGS

Basic drugs include the following: amphetamines, phencyclidine, proxyphene, meperidine, LSD, codeine, oxycodone, opiates.

Although very different in their structure, all basic drugs feature an amine functional group (NR_3 , NR_2H , or NRH_2). This group acts as a base by abstracting H^+ and becoming positively charged. Initial extraction, however, takes place by a nonpolar mechanism onto the hydrophobic portion of the sorbent. After the drug is retained, washing the cartridge with water removes polar interferences. Next, the cartridge is washed with acid, completing the elution of polar interferences and ensuring that the basic drugs are positively charged as ammonium salts. Nonpolar, non-basic drugs and interferences can then be removed with an organic wash. The presence of water during the organic wash negatively affects the efficiency of the wash by minimizing contact of the sorbent with the organic solvent. Therefore, it is important that the sorbent is thoroughly dried to remove any residual water before the organic wash. Finally, the basic drugs can be eluted with an alkaline organic solvent (i.e. 2% NH_4OH in either methanol, ethyl acetate (EtOAc), or methylene chloride/isopropyl alcohol ($\text{CH}_2\text{Cl}_2/\text{IPA}$)). The presence of base disrupts the ionic interactions of the drug with the sorbent by neutralizing the positive charge on the drug. The use of organic solvent disrupts the hydrophobic interactions, which initially retained the drug from the sample.

Section 4 - ACIDIC AND NEUTRAL DRUGS

As with basic drugs, acidic and neutral drugs have widely varying structural properties. They are classed together because they are not retained by a cation-exchange mechanism, although the cation-exchange portion of Bond Elut Certify can improve cleanup of samples containing these drugs. These drugs are characterized by the absence of a basic amine functional group. (Drugs such as barbiturates contain a nitrogen-containing *imine* functional group, which is weakly acidic, rather than basic.) These drugs are retained on Bond Elut Certify by a nonpolar mechanism. Washing the cartridge with dilute acid removes polar impurities, and ensures that any basic interferences become charged. Thus, when the acidic and neutral drugs are eluted by disrupting their nonpolar interaction with the sorbent, the basic interferences are retained on the strong cation-exchange portion of the sorbent.

Bond Elut Certify II is a mixed mode sorbent originally developed for the tetrahydrocannabinol (THC) metabolite Δ^9 -carboxy THC. Because this drug contains an acidic functional group, cleanup from urine samples can be optimized by using an *anion*-exchange sorbent rather than the cation-exchanger found in Bond Elut Certify. As with basic drugs on Bond Elut Certify, retention of acidic drugs on Bond Elut Certify II is initially achieved by nonpolar interactions on the hydrophobic portion of the sorbent. Next, polar interferences can be washed away with a basic buffer. This wash step also ensures that the COOH functional group is deprotonated, forming COO⁻, which can then be retained on the anion-exchange portion of the Bond Elut Certify II sorbent. The charge on any amine functional groups would be neutralized by this step as well, preparing any basic drugs present for washing. After briefly drying the cartridge, nonpolar basic drugs and interferences can be removed with a nonpolar solvent. Finally, the Δ^9 -carboxy THC can be recovered by elution with a nonpolar acidic solvent such as hexane/ethyl acetate with 1% acetic acid.

BOND ELUT CERTIFY AND CERTIFY II

Method Optimization

Many of the methods contained in this booklet have been successfully employed by the largest workplace testing laboratories throughout the world for over 20 years.

Many laboratories modify these methods to suit their specific needs or requirements. In addition, with the advent of more sensitive detectors, the desire to analyze additional drug metabolites, the need for increased throughput, and advancements in chromatography techniques, further modifications are possible.

The following information should aid in making modifications to these methods to suit specific analytical requirements. Should you have additional questions about these methods or modification considerations, please contact your local Agilent Support Representative at agilent.com/chem/contactus.

Cartridge Bed Mass

These methods and modifications are designed for the Bond Elut Certify and Certify II cartridge. Standard Bond Elut Certify methods in this manual (strong cation-exchange and C8) use a 130 mg bed mass. Bond Elut Certify II (strong anion-exchange and C8) uses a 200 mg bed mass. Smaller sample sizes can lead to the use of smaller bed masses and in turn, lower rinse and elution volumes, and potentially increase throughput. Agilent's technical support team would be pleased to discuss these options with you.

Sample Size

With significant improvements in chromatography and detector technology, a 5 mL urine sample is typically no longer necessary – 0.5-3 mL is often sufficient for typical levels of detection. The volume of accompanying buffer can be proportionally altered, or for ease-of-use, remain the same at 2 mL buffer. Typical sample volumes are 2 mL of urine matrix with 2 mL buffer.

Conditioning

2 mL methanol (MeOH) followed by 2 mL of buffer are still considered standard for conditioning. Labs sometimes use gravity flow at this step to ensure that the SPE beds do not go to dryness. To facilitate faster methods or reduce solvent usage, 500 μ L of MeOH can be used. Apply the MeOH with approximately 2 inches Hg vacuum until the MeOH has entered the column completely. Gravity may also be used. Follow the MeOH with 1 mL of specified buffer and allow the buffer to pass through the cartridges. Because the buffer is also used to dilute the urine samples, residual buffer above the top frit of the SPE cartridge is acceptable. *Avoid high levels of vacuum and extensive drying times prior to the addition of the sample. This can cause de-conditioning of the cartridge and can affect recoveries.* When working with serum/plasma samples, 1-2 mL buffer is a typical volume for removing residual MeOH in the conditioning step. This prevents protein precipitation as the sample passes through the cartridge.

Column Washing and Drying

Methods may be modified such that no individual rinse step is more than 3 mL. Note that drying the cartridges is often recommended between column washes or prior to elution. In some cases, such as THC and its metabolite, over-drying can lead to diminished recoveries. For THC, 10-15 inches Hg of vacuum for 2-3 minutes is recommended where noted in the method. For most methods, drying prior to elution facilitates the rapid elution of compounds from the cartridge and is recommended.

Elution

Unless specified, 2 mL is an optimal volume. To optimize recovery, elution should be performed in multiple aliquots (2 x 1 mL). This does increase the number of steps required in the method.

Derivatization

The options for the types of derivatives are too numerous to mention here. BSTFA is a very rugged and often-used derivatization reagent. Other derivatives are employed to move peaks of interest away from interfering peaks, thus maintaining ion ratios. Derivatives are also used to increase compound mass and sensitivity with MS detectors.

Alternative Matrices

Hair, oral fluids and tissue matrices are routinely tested in many laboratories. Agilent offers analytical approaches to these alternative samples. Visit agilent.com/chem/forensics for more information.



BOND ELUT CERTIFY AND CERTIFY II

Part Numbers

Bond Elut Certify and Certify II are available in a wide variety of cartridge volumes to accommodate both manual and automated drug extraction methods. Sorbent masses from 50 mg to 1 g are available for both standard drug testing methods and pharmaceutical research.

Bond Elut Certify and Certify II are also available in 96-well plate formats. These formats include our 1 mL 96-well plate, 2 mL 96-square well plate, and the VersaPlate format. The VersaPlate format incorporates 96 individual SPE cartridges that can be assembled into a 96-well array, using a reusable holder, or the VersaPlate tubes function as standalone SPE cartridges for vacuum or positive pressure processing. The VersaPlate array format minimizes waste, ensures high throughput, and is ideal for scalability in your methods.

Bond Elut Certify

Description	Unit	40 μ m Particle Size	120 μ m Particle Size
Large Reservoir Capacity (LRC) Cartridges			
130 mg, 10 mL	50/pk	12113050	14113050
130 mg, 10 mL	500/pk	52113050	14113055
200 mg, 10 mL	50/pk	12113054	14113054
200 mg, 10 mL	500/pk	52113051	
300 mg, 10 mL	50/pk	12113052	14113052
Straight Barrel Cartridges			
50 mg, 3 mL	50/pk	12105030	
130 mg, 1 mL	100/pk	12102083	14102083
130 mg, 3 mL	50/pk	12102051	14102051
130 mg, 3 mL	500/pk	52102051	
130 mg, 3 mL ASPEC	500/pk	22102084	
130 mg, 3 mL tabless	50/pk	12102051T	
130 mg, 6 mL	30/pk	12256146	
130 mg, 6 mL tabless	500/pk	12256146TJ	
200 mg, 3 mL	50/pk	12102145	
200 mg, 6 mL	30/pk	12256145	
300 mg, 3 mL	50/pk	12102081	
300 mg, 3 mL	500/pk	52102081	
300 mg, 3 mL ASPEC	500/pk	22102081	
300 mg, 3 mL tabless	50/pk	12102081T	14102081T
300 mg, 6 mL	30/pk	12102082	
500 mg, 6 mL	30/pk	12102093	14102093
1 g, 6 mL	30/pk	12102085	14102085

TIP: To meet your lab's high-throughput needs, choose cost-effective bulk packaging for many of our products. Visit [agilent.com/chem/bigpacks](https://www.agilent.com/chem/bigpacks)

Bond Elut Certify VersaPlate Formats

Description	Particle Size (µm)	25 mg	50 mg	100 mg
Preassembled 96-well plate	40		75409050	7540901C
VersaPlate tubes*	40	75509025	75509050	7550901C

*Tubes need to be inserted into a VersaPlate base plate, P/N 75400000

Bond Elut Certify 96-well Plates

Description	25 mg	50 mg	100 mg
1 mL round-well plates	A4960925	A4960950	A496091C
2 mL square-well plates	A3960925	A3960950	A396091C

Bond Elut Certify II

Description	Unit	40 µm Particle Size	120 µm Particle Size
Large Reservoir Capacity (LRC) Cartridges			
100 mg, 10 mL	50/pk	12113063	
200 mg, 10 mL	50/pk	12113051	14113051
Straight Barrel Cartridges			
50 mg, 3 mL	50/pk	12105031	
100 mg, 1 mL	100/pk	102818C	
200 mg, 3 mL	50/pk	12102080	14102080
500 mg, 6 mL	30/pk	12102084	14102084
1g, 6 mL	30/pk	12102088	14102088

Bond Elut Certify II VersaPlate Formats

Description	Particle Size (µm)	100 mg
Preassembled 96-well plate	40	7541801C
VersaPlate tubes*	40	7551801C

*Tubes need to be inserted into a VersaPlate base plate, P/N 75400000

Bond Elut Certify II 96-well Plates

Description	25 mg	50 mg	100 mg
1 mL round-well plates	A4961825	A4961850	A496181



TIP: To view a demonstration of the VersaPlate format, visit agilent.com/chem/versaplatevideo



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SOLVENTS, SOLVENT MIXTURES, REAGENTS, AND SOLUTIONS USED IN BOND ELUT CERTIFY AND CERTIFY II EXTRACTIONS OF DRUGS OF ABUSE

SECTION 1

SOLVENTS

Acetone: HPLC Grade
Acetonitrile (CH₃CN): HPLC Grade
Chloroform (CHCl₃): HPLC Grade
Distilled or Deionized Water (DI H₂O): 5 ≤ pH ≤ 7
Ethyl Acetate (EtOAc): HPLC Grade
Hexane: HPLC Grade
Isopropyl Alcohol (IPA): HPLC Grade
Methanol (CH₃OH or MeOH): HPLC Grade
Methylene Chloride (CH₂Cl₂ or MeCl₂): HPLC Grade

SECTION 2

SOLVENT MIXTURES

Acetone/Chloroform (50/50)
Acetonitrile/DI H₂O (25/75)
CH₂Cl₂/IPA (80/20)
Hexane/Ethyl Acetate (50/50)
Hexane/Ethyl Acetate (80/20)
Methanol/DI H₂O (10/90)

NOTES:

Storage of organic solvents in some plastic containers may lead to contamination of the solvent or solvent mixture by plasticizers, which may interfere with analyte quantitation. Good laboratory practice dictates that those who handle or are potentially exposed to reagents, solvents, and solutions used or stored in the laboratory should familiarize themselves with manufacturer's recommendations for chemical storage, use, and handling, and should also familiarize themselves with an appropriate Safety Data Sheet (SDS) for each material for which a Safety Data Sheet exists.

TIP: Easy, Reliable pH Testing, Designed for Chromatographers.

Agilent now offers a full line of pH meters and electrodes.

Designed for chromatographers, these pH meters offer intuitive user design and exceptional ruggedness for your lab. Learn more at

agilent.com/chem/phmeters

SECTION 3

REAGENTS

Acetic Acid, Glacial (CH_3COOH): 17.4 M
Ammonium Hydroxide (NH_4OH): concentrated (14.8 M)*
 β -Glucuronidase: lyophilized powder from limpets (*Patella Vulgatta*)
Dimethylformamide (DMF): silylation grade
Hydrochloric Acid (HCl): concentrated (12.1 M)
N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) with 3% trimethylsilyliodide
N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS)
Pentafluoropropionic Acid Anhydride (PFPA)
Phosphoric Acid (H_3PO_4): concentrated (14.7 M)
Potassium Bicarbonate (KHCO_3): F.W. 100.12
Potassium Hydroxide (KOH): F.W. 56.11
Potassium Phosphate Monobasic (KH_2PO_4): F.W. 136.09
Sodium Acetate (CH_3COONa): F.W. 82.03
Sodium Acetate Trihydrate ($\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$): F.W. 136.08
TRIS Base

*Note: For best performance, order ammonium hydroxide in small volume bottles. Store capped and minimize opening and closing of the reagent. Fresh ammonium hydroxide is very important for consistent recoveries.

SECTION 4

SOLUTIONS AND BUFFERS

Acetic Acid, 1.0 M:

To 400 mL DI H_2O , add 28.6 mL glacial acetic acid. Dilute to 500 mL with DI H_2O . Mix.
storage: 25 °C in glass or plastic
stability: 6 months

Acetic Acid, 0.01 M:

Dilute 57.5 μL glacial acetic acid to 100 mL with DI H_2O . Mix.
storage: 25 °C in glass or plastic
stability: 6 months

Acetic Acid, 100 mM:

Dilute 40 mL 1.0 M acetic acid to 400 mL with DI H_2O . Mix.
storage: 25 °C in glass or plastic
stability: 6 months

SOLUTIONS AND BUFFERS *continued***Acetate Buffer, 100 mM (pH 4.0):**

Into a 100 mL volumetric flask, add 80 mL DI H₂O; to this, add 570 µL of glacial acetic acid. Mix. Add 1.6 mL of 1.0 M KOH. Check pH. The pH should be 4.0. Adjust the pH to 4.0 if necessary. Make up to volume with DI H₂O. Mix well.

storage: 25 °C in glass or plastic

stability: 6 months; Inspect daily with use for contamination

Acetate Buffer, 1.0 M (pH 5.0):

Dissolve 42.9 g sodium acetate trihydrate in 400 mL DI H₂O; add 10.4 mL glacial acetic acid. Dilute to 500 mL with DI H₂O. Mix. Adjust pH to 5.0 ± 0.1 with 1.0 M sodium acetate or 1.0 M acetic acid.

storage: 25 °C in glass or plastic

stability: 6 months; Inspect daily with use for contamination.

β-Glucuronidase, 5,000 Fishman units/mL:

Dissolve 100,000 Fishman units lyophilized powder with 20 mL acetate buffer, 1.0 M (pH 5.0).

storage: -5 °C in plastic

stability: Several days; prepare daily for best results.

Ethyl Acetate/Ammonium Hydroxide (98/2):

To 98 mL EtOAc, add 2 mL concentrated NH₄OH. Mix.

storage: 25 °C in glass

stability: 1 day

Hydrochloric Acid, 100 mM:

To 400 mL DI H₂O, add 4.2 mL concentrated HCl. Dilute to 500 mL with DI H₂O. Mix.

storage: 25 °C in glass or plastic

stability: 6 months

Hydrochloric Acid, 1.0 M:

Into a 100 mL volumetric flask, add 50 mL DI H₂O. To this, add 8.3 mL of concentrated HCl. Bring to volume with DI H₂O.

storage: 25 °C in glass

stability: 1 day

Methylene Chloride/Isopropanol/Ammonium Hydroxide (78/20/2):

To 20 mL IPA, add 2 mL concentrated NH₄OH. Mix. Add 78 mL CH₂Cl₂. Mix.

storage: 25 °C in glass or fluoropolymer plastic

stability: 1 day

Phosphate Buffer, 100 mM (pH 6.0):

Weigh 13.6 g of KH_2PO_4 into a 1.0 L volumetric flask. Dissolve the KH_2PO_4 into 900 mL DI H_2O . Adjust pH to 6.0 (± 0.1) with 1.0 M KOH while stirring. Bring the total volume up to 1.0 L with DI H_2O .

storage: 5 °C in glass

stability: 1 month; Inspect daily with use for contamination.

Phosphoric Acid, 50 mM:

Add 3.4 mL of phosphoric acid to 950 mL DI H_2O in a 1.0 L volumetric flask. Mix and bring to volume with DI H_2O .

storage: 25 °C in glass or plastic

stability: 6 months

Potassium Hydroxide, 1.0 M:

Weigh 5.6 g KOH into a clean plastic 100 mL volumetric flask. Dissolve the KOH with DI H_2O and bring to volume.

storage: 25 °C in plastic

stability: 6 months

Potassium Hydroxide, 10.0 M:

Into a 250 mL plastic volumetric flask, add 150 mL DI H_2O . To this, add 140 g KOH.

Dissolve the KOH and bring to 250 mL with DI H_2O .

storage: 25 °C in plastic

stability: 3 months

Sodium Acetate, 1.0 M:

Dissolve 13.6 g sodium acetate in 90 mL DI H_2O . Dilute to 100 mL with DI H_2O . Mix.

storage: 25 °C in glass or plastic

stability: 6 months

TRIS Buffer, 2.0 M (pH 8.1):

Weigh 242.2 g TRIS base (tris[hydroxymethyl]aminomethane) in a 1.0 L volumetric flask.

Dissolve TRIS base* in 900 mL DI H_2O . Adjust the pH to 8.1 with 1.0 M HCl while stirring. Bring the total volume to 1.0 L with DI H_2O . TRIZMA* HYDROCHLORIDE (tris[hydroxymethyl]aminomethane hydrochloride) can also be used to prepare the buffer.

A 2.0 M solution requires 315.2 g of TRIZMA HCl, and NaOH is required to bring the acidic solution to a pH of 8.1.

storage: 25 °C in glass or plastic

stability: 30 days

*NOTE: TRIS base is also known as TRIZMA base or THAM.

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VAC ELUT VACUUM EXTRACTION MANIFOLDS

- Increased productivity and sample throughput
- Disposable needles eliminate cross contamination
- Rugged, reliable construction

Engineered to increase laboratory productivity, the reliable family of Vac Elut vacuum extraction manifolds supports extraction of up to 12, 20, or 24 samples at one time, for improved efficiency. Vac Elut manifolds are offered in traditional glass-basin manifolds in 12 or 20-position configurations. This manifold style offers corrosion resistance and easy visualization of the extraction process.

The Vac Elut SPS 24 is a unique design that supports up to 24 sample extractions, good for higher throughput applications.

Select the right manifold for your extraction needs. We also offer a range of spare parts and accessories for our cartridge manifolds to maintain performance.



VAC ELUT SPS 24 MANIFOLD

- Closed operation prevents cross contamination
- Stainless steel tips deliver maximum extract purity
- Range of rack sizes covers most tube configurations

The Vac Elut SPS 24 allows simultaneous processing of up to 24 SPE cartridges. Like all Vac Elut manifolds, the SPS 24 is made from durable, solvent-resistant materials and engineered to last. The glass sides allow easy viewing of the entire sample collection process.

The ultimate feature of the SPS 24 manifold is its waste diversion funnel, which enables all steps of the SPE procedure to be completed without removing the lid. Since the collection rack is placed inside the unit before extraction begins, splash back and cross contamination are eliminated, while hazardous waste and biohazard exposure are minimized. Wastes collect outside of the manifold itself, simplifying cleanup and reducing the time needed to extract and elute samples.

Complete with replacement stainless steel delivery tips for maximum extract purity, the Vac Elut SPS 24 system also includes a vacuum controller/release, collection rack, and port sealing plugs. Racks for several different collection tube configurations are available.

Vac Elut SPS 24 Manifold

Description	Part No.
Vac Elut SPS 24 manifold with collection rack for 10 x 75 mm test tubes	12234003
Vac Elut SPS 24 manifold with collection rack for 12 x 75 mm test tubes	12234041
Vac Elut SPS 24 manifold with collection rack for 13 x 100 mm test tubes	12234022
Vac Elut SPS 24 manifold with collection rack for 16 x 100 mm test tubes	12234004
Replacement Components	
Collection rack and funnel set for 12 or 15 mL conical tubes	12234027
Collection rack and funnel set for 12 x 75 mm test tubes	12234030
Collection rack and funnel set for 13 x 100 mm test tubes	12234031
Collection rack and funnel set for 16 x 100 mm test tubes	12234028
Elastic lid fasteners, 6/pk	12234034
Complete upper lid assembly	12234025C
SPS 24 upper lid cover	12234025
SPS 24 waste tower repair kit Includes base exit tube, hose connector, washer, center tube, 900 connector elbow	12234005
Stainless steel delivery needles, 25/pk	12234038

VAC ELUT 20 VACUUM EXTRACTION MANIFOLDS

The Vac Elut 20 is a glass-basin manifold with a 20-sample capacity. For ease of use, the vacuum control valve, vacuum gauge, and quick release valve are mounted on the lid, away from the corrosive waste stream and within convenient reach. The solvent-resistant polypropylene rack is available in a variety of sizes to accommodate collection tubes commonly used in sample preparation. Manifold sets include the glass basin, lid cover, collection rack and vacuum gauge assembly.

Vac Elut 20 Manifold

Description	Part No.
Manifold Sets	
Vac Elut 20 manifold with collection rack for 10 x 75 mm test tubes	12234105
Vac Elut 20 manifold with collection rack for 13 x 75 mm test tubes	12234100
Vac Elut 20 manifold with collection rack for 13 x 100 mm test tubes	12234101
Vac Elut 20 manifold with collection rack for 16 x 75 mm test tubes	12234102
Vac Elut 20 manifold with collection rack for 16 x 100 mm test tubes	12234103
Accessories for Vac Elut 20 Manifold	
Standard glass basin	12234505
Collection rack for 10 x 75 mm test tubes	12234517
Collection rack for 13 x 75 mm test tubes	12234507
Collection rack for 16 x 100 mm test tubes	12234510
Replacement Components	
Polypropylene delivery needles, 25/pk	12234511
Replacement exit valve for glass basin	12234506
Replacement lid gasket	12234502
Vac Elut 20 lid cover	12234501
Vacuum gauge assembly	12234504

VAC ELUT 20 MANIFOLD WITH TALL GLASS BASIN

- For extractions greater than 10 mL
- Transparent glass base allows you to monitor the whole collection operation
- Simple vacuum adjustment

The Vac Elut 20 with a large glass basin and collection rack accommodates larger 16 x 150 mm test tubes. The same high quality material and features on the standard Vac Elut system are incorporated on this special unit. These collection vessels can be utilized in combinatorial chemistry applications using large boiling tubes for collection of purified synthesis mixtures, or for any SPE extraction in which an elution volume greater than 10 mL is required.

Vac Elut 20 Manifold with Tall Glass Basin

Manifold Set	Part No.
Vac Elut 20 Manifold with tall glass basin and collection rack for 16 x 150 mm test tubes, complete system	12234104



For more information, visit [agilent/chem/sampleprep](https://www.agilent.com/chem/sampleprep)

VAC ELUT 12 MANIFOLD

The Vac Elut 12 vacuum extraction manifold is a compact tool for small sample sets. The Vac Elut 12 offers the same durability of components and operation as the Vac Elut 20 manifolds, but works well when only a few samples need to be processed at a time. The Vac Elut has 12 sample positions, a clear glass basin for easy visualization of the extraction, and a gauge for precise vacuum settings.



Vac Elut 12 Manifold

Manifold Set	Part No.
Vac Elut 12 manifold with collection rack for 16 x 100 mm test tubes	5982-9110

Replacement Parts for Vac Elut Vacuum Manifolds

Description	Part No.
Manifold ball ring/vacuum quick release	5982-9106
Manifold exit valve replacement kit	5982-9107
Manifold vacuum gauge assembly with valve	5982-9108
White cover for 12-port manifold	5982-9111
Sealing gasket for 12-port manifold	5982-9112
Glass chamber for 12-port manifold	5982-9113
12-port rack for 13 x 75 mm tubes	5982-9114
12-port rack for 13 x 100 mm tubes	5982-9115
12-port rack for 16 x 75 mm tubes	5982-9116
12-port rack for 16 x 100 mm tubes	5982-9117

Parts and Disposables for Vac Elut Cartridge Manifolds

Description	Unit	Part No.
Disposable needle tip	20/pk	5982-9100
Stainless steel needle with polypropylene coating	20/pk	5982-9101
Short valve stopcock	20/pk	5982-9102
Long valve stopcock	20/pk	5982-9103
Male luer plugs	25/pk	5982-9104
Needle tip ejector tool		5982-9105
Cartridge stacking adapters	12/pk	5982-9109

AGILENT 96-WELL PLATE VACUUM MANIFOLDS AND ACCESSORIES

To support 96-well plate extraction workflows, Agilent offers two main configurations of vacuum manifold. The Vac Elut 96-well plate manifold has a small footprint, comes with an on/off valve, and a vacuum control valve. The Vac Elut 96 is designed for use with Bond Elut 2-mL square-well plates. Spacer inserts support different collection plate configurations. An adapter ring can be used to modify the manifold for use with 1 mL plates. For our VersaPlate configuration, we offer the VersaPlate manifold, which is the ideal companion for our VersaPlate SPE products.

A range of collection plates and seals are also offered for use in 96-well plate sample preparation workflows.

Agilent Vacuum Manifold for 96-well Plates

Description	Part No.
Manifold for 96-well plates Includes base, vacuum gauge, needle valve and fixed lid	5185-5776

Parts and Accessories for Agilent 96-well Plate Manifold

Description	Part No.
Base O-ring for Agilent 96-well plate manifold	5185-5779
Collection plate spacer for Agilent 1 mL deep-well, 12 mm	5185-5775
Collection plate spacer for microtiter plate and Agilent 0.5 mL shallow well plate, 29 mm	5185-5781
Collection plate spacer for most industry-standard deep-well collection plates, 2 mm	5185-5780
Agilent 96-well vacuum manifold, base assembly only	5185-5797
Lid for Agilent 96-fixed well vacuum manifold	5185-5798
Lid gasket for Agilent 96-well plate manifold	5185-5778
Adapter for deep-profile (1 mL round) SPE plates	A596AD
Needle valve for Agilent 96-well manifold	5185-5783
On/off valve for Agilent 96-well manifold	5185-5785
Vacuum gauge for Agilent 96-well manifold	5185-5786
Vacuum outlet (Ni plated) for Agilent 96-well manifold	5185-5784

Agilent Bond Elut 96-well Accessories

Description	Unit	Part No.
96-well manifold, shimset	1/pk	12236104
Square-well collection plates, 2 mL	50/pk	5133009
Square-well collection plates, 1 mL	50/pk	5133008
Square-well collection plates, 350 µL	50/pk	5133007
Sealing tape pad	10/pk	12143105
Square 96-well sealing caps, EVA, pierceable	50/pk	5133005
VersaPlate sealing strips, each covers one column	240/pk	12236108
Captiva 96-deep well collection plates, 1 mL	10/pk	A696001000
Disposable reservoir trays for 96-well manifold	25/pk	5185-5782
Round-well collection plates, 1 MI	50/pk	5132101
Square-well collection plates, 350 µL	50/pk	5133007
Square-well collection plates, 1 mL	50/pk	5133008
Square-well collection plates, 2 mL	50/pk	5133009

Agilent VersaPlate Manifold and Accessories

Description	Part No.
VersaPlate vacuum manifold, complete	12236101
Base Plate, empty	75400000
VersaPlate manifold, acetal, white, bottom	12236103
VersaPlate manifold, bottom replacement gasket	12236105
VersaPlate sealing strips	12236108
VersaPlate tube removal tool	12236107



For more information, visit [agilent/chem/sampleprep](http://agilent.com/chem/sampleprep)

BOND ELUT ADAPTERS

- Connect SPE cartridges in series for large samples
- Expand cartridge volume for even more applications
- Transfer large-volume samples to any SPE cartridge

Bond Elut Adapters

Description	Unit	Part No.
Adapter cap for 1, 3 and 6 mL Bond Elut cartridges	15/pk	12131001
Adapter cap for LRC 12, and 20 mL Bond Elut cartridges	10/pk	12131003
Adapter cap for 60 mL Bond Elut cartridges	10/pk	12131004

Bond Elut adapters fit on top of any Bond Elut cartridge and contain a female Luer fitting that accommodates the tip of another cartridge, allowing the following configurations:

Bond Elut Adapter Configurations

Configuration 1: Stack two cartridges to perform multi-sorbent methods

Configuration 2 + 3: Increase any cartridge's volume by stacking an empty reservoir on top of the device.

Configuration 4: Standard Luer-tipped syringes will fit into any Bond Elut adapter. Gentle pressure can then be used to apply conditioning solvents, samples, rinsing solvents and eluents. This configuration is particularly useful for single sample processing, where a vacuum manifold is not required.

Configuration 5: For excessively large sample volumes, 1/8 in od tubing can be connected to the end of an adapter and the sample can be drawn directly from the sample container via high vacuum.



LUER STOPCOCKS

- Control flow rates during SPE vacuum extraction
- Improve method reproducibility
- Instant isolation from vacuum reduces accidental tube drying

Luer stopcocks are used to provide independent flow control of each individual Bond Elut cartridge when used with vacuum manifolds. They are made from solvent-resistant high-grade polypropylene, are reusable and can be readily cleaned using organic solvents such as methanol or acetone.

Luer Stopcocks

Description	Unit	Part No.
Luer stopcocks	15/pk	12131005

ADAPTER CAPS FOR GILSON ASPEC SPE SYSTEMS

- Enhance the high-throughput compatibility of Bond Elut cartridges
- Converts 1, 3 and 6 mL cartridges for use in Gilson SPE systems
- Specially engineered for leak-free operation

Gilson-engineered caps produce a positive pressure seal with the needle in Gilson ASPEC, ASPEC XL and ASPEC XL4 solid phase extraction systems.

Adapter Caps for Gilson ASPEC SPE Systems

Description	Unit	Part No.
Gilson adapter cap, 1 mL	1000/pk	12131034
Gilson adapter cap, 3 mL	1000/pk	12131035
Gilson adapter cap, 6 mL	1000/pk	12131036



RESOLVE YOUR SEARCH FOR SENSITIVITY, SPEED, AND SEAMLESS INTEGRATION

Agilent 5977A Series GC/MSD builds on a 45-year tradition of leadership and innovation, bringing together the technologies of the industry's best GC and MS systems.

This advanced instrument provides everything you need to take your lab to a higher plane of productivity and confidence – including increased sensitivity, superior workflow, and software tools that simplify method optimization and lower your operating costs.

Simply put, the 5977A Series GC/MSD can help you meet your toughest challenges... today and in the future.

- **Higher sensitivity** – Our Extractor Ion source and tuning protocols increase MSD sensitivity, so you can confidently detect trace-level compounds and routinely achieve lower detection limits.

- **Maximum productivity** – The integrated hardware and software features simplify your workflow and help you get more done with fewer resources.

- **The best software options** – You pick the workflow that makes you most productive. Continue to use our versatile and robust ChemStation – or choose our proven MassHunter software.

ENSURE A RELIABLY INERT FLOW PATH – AND IMPROVE RESPONSE WITH ACTIVE ANALYTES

By minimizing activity along every step of the GC and GC/MS flow path, Agilent Inert Flow Path solutions help ensure an inert GC flow path for higher sensitivity, accuracy, linearity, and reproducibility, especially at trace levels. They also minimize the need for frequent inlet maintenance and system recalibration.

AGILENT ULTRA INERT LINERS AND FERRULES

Agilent Ultra Inert liners and touchless packaging

Description	Volume (µL)	ID (mm)	1/pk	5/pk	25/pk	100/pk*
Splitless Inlet Liners						
Single taper, Ultra Inert Liner	900	4	5190-2292	5190-3162	5190-3166	5190-3170
Single taper, Ultra Inert Liner with glass wool	900	4	5190-2293	5190-3163	5190-3167	5190-3171
Double taper, Ultra Inert liner	800	4	5190-3983	5190-4007		
Dimpled	200	2	5190-2297	5190-4006		
Straight			5190-6168			
Split Inlet Liners						
Straight, Ultra Inert Liner with glass wool	990	4	5190-2294	5190-3164	5190-3168	5190-3172
Low pressure drop, Ultra Inert Liner with glass wool	870	4	5190-2295	5190-3165	5190-3169	5190-3173
SPME, Headspace Injection						
Straight Ultra Inert Liner for SPME	35	0.75	5190-4048			
Straight, Ultra Inert Liner	65	1	5190-4047			

*The 100/pk is not in the Touchless packaging. O-rings must be purchased separately.

Go back to [Table of Contents](#)

Agilent UltiMetal Plus Flexible Metal ferrules

Description	Unit	Part No.
UltiMetal Plus Flexible Metal ferrule with 0.4 mm id. For fused silica tubing 0.1-0.25 µm id	10/pk	G3188-27501
UltiMetal Plus Flexible Metal ferrule with 0.5 mm id. For fused silica tubing 0.32 µm id	10/pk	G3188-27502
UltiMetal Plus Flexible Metal ferrule with 0.8 mm id. For fused silica tubing 0.45-0.53 µm id	10/pk	G3188-27503
UltiMetal Plus Flexible Metal ferrule with no hole. To plug Capillary Flow Technology fittings	10/pk	G3188-27504
UltiMetal Plus Flexible Metal ferrule. Use with 0.25 mm and 0.32 mm UltiMetal column tubing	10/pk	G3188-27505
UltiMetal Plus Flexible Metal ferrule. Use with 0.53 mm UltiMetal column tubing	10/pk	G3188-27506

AGILENT J&W ULTRA INERT GC COLUMNS

HP-1ms Ultra Inert

ID (mm)	Length (m)	Film (µm)	Temp Limits (°C)	Part No.
0.18	20	0.18	-60 to 325/350	19091S-677UI
0.25	15	0.25	-60 to 325/350	19091S-931UI
		0.25	-60 to 325/350	19091S-933UI
	30	0.50	-60 to 325/350	19091S-633UI
		1.00	-60 to 325/350	19091S-733UI
0.32	15	0.25	-60 to 325/350	19091S-911UI
		0.52	-60 to 325/350	19091S-612UI
	30	0.25	-60 to 325/350	19091S-913UI
		1.00	-60 to 325/350	19091S-713UI

HP-5ms Ultra Inert

ID (mm)	Length (m)	Film (µm)	Temp Limits (°C)	Part No.
0.18	20	0.18	-60 to 325/350	19091S-577UI
0.25	15	0.25	-60 to 325/350	19091S-431UI
		0.25	-60 to 325/350	19091S-433UI
	30	0.50	-60 to 325/350	19091S-133UI
		1.00	-60 to 325/350	19091S-233UI
0.32	60	0.25	-60 to 325/350	19091S-436UI
		0.25	-60 to 325/350	19091S-413UI
	30	1.00	-60 to 325/350	19091S-213UI

DB-5ms Ultra Inert

ID (mm)	Length (m)	Film (µm)	Temp Limits (°C)	Part No.
0.18	20	0.18	-60 to 325/350	121-5522UI
		0.36	-60 to 325/350	121-5523UI
0.25	15	0.25	-60 to 325/350	122-5512UI
		1.00	-60 to 325/350	122-5513UI
	25	0.25	-60 to 325/350	122-5522UI
		0.25	-60 to 325/350	122-5532UI
	30	1.50	-60 to 325/350	122-5536UI
		1.00	-60 to 325/350	122-5533UI
0.32	50	0.25	-60 to 325/350	122-5552UI
		0.25	-60 to 325/350	122-5562UI
	60	1.00	-60 to 325/350	122-5563UI
		30	0.25	-60 to 325/350
0.32	30	0.50	-60 to 325/350	123-5536UI
		1.00	-60 to 325/350	123-5533UI
	60	1.00	-60 to 325/350	123-5563UI

DB-35ms Ultra Inert

ID (mm)	Length (m)	Film (µm)	Temp Limits (°C)	Part No.
0.18	20	0.18	50 to 340/360	121-3822UI
0.25	15	0.25	50 to 340/360	122-3812UI
		0.25	50 to 340/360	122-3832UI
0.32	30	0.25	50 to 340/360	123-3832UI

For more information, visit agilent/chem/sampleprep

INFINITELY MORE CONFIDENT

The Agilent 1260 Infinity LC raises the standard in HPLC – without raising the price. It offers new levels of productivity, data quality and robustness to give you highest confidence in your investment.

- 600 bar standard pump pressure, 80 Hz standard detector speed and up to 10 times higher UV detection sensitivity – be prepared for the challenges of today and tomorrow
- 100% compatible with all your HPLC methods – ensuring riskless replacement of existing equipment.

INFINITELY MORE SENSITIVE

The Agilent 6460 Triple Quadrupole LC/MS System is equipped with Agilent Jet Stream Technology to provide ultra-sensitive performance for analytes in the most complex matrices, making it the instrument of choice for a wide range of applications. The 6460 provides a fast and robust solution for simultaneously quantifying, screening, and confirming analytes using triggered MRM (tMRM). It is engineered to deliver robust, accurate quantitation of analytes present at extremely low levels. Updated electronics enable high speed data acquisition while fast polarity switching makes the instrument an ideal choice for rapid separations and high-throughput analyses of diverse compound types.



AGILENT POROSHELL 120 COLUMNS

LC/MS methods are rapidly becoming an important tool for drug screening and confirmation. Poroshell 120 columns provide exceptional efficiency for standard HPLC, and significantly boost the performance you'll get from UHPLC instruments.

We recommend you start your method development with Poroshell 120 EC-C18 as it will provide the best results across a broad range of analytes.

Recommended columns for Forensic Toxicology separations

Size (mm)	Poroshell 120 EC-C18	Poroshell 120 EC-C8
3.0 x 150	693975-302	693975-306
3.0 x 100	695975-302	695975-306
3.0 x 75	697975-302	697975-306
3.0 x 50	699975-302	699975-306
3.0 x 30	691975-302	691975-306
2.1 x 150	693775-902	693775-906
2.1 x 100	695775-902	695775-906
2.1 x 75	697775-902	697775-906
2.1 x 50	699775-902	699775-906
2.1 x 30	691775-902	691775-906
Fast Guard 3.0 x 5	823750-911	823750-913
Fast Guard 2.1 x 5	821725-911	821725-913

There are nine different phases in the Poroshell 120 family, and 4.6 mm id columns are available as well.

For a full list of part numbers, visit agilent.com/chem/poroshell120



BOND ELUT CERTIFY EXTRACTION METHODS

INTRODUCTION AND OVERVIEW
OF THE MANUAL

SOLVENTS, SOLVENT MIXTURES,
REAGENTS, AND SOLUTIONS

EQUIPMENT AND ACCESSORIES

BOND ELUT CERTIFY
EXTRACTION METHODS

BOND ELUT CERTIFY II
EXTRACTION METHODS

APPENDIX

ANALYTE NAME/MATRIX

M2707A

Amphetamines in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both nonpolar and cation-exchange mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 2 mL of urine, add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 0.1 M phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 1 mL 1.0 M acetic acid; draw through under vacuum
- Dry column for 5 minutes under vacuum
- 2 mL CH₃OH; draw through under vacuum
- Dry column (2 minutes at ≥ 10 inches Hg)

4. Elution

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily

5. Concentrate Eluate

- Add 100 μ L silylation grade DMF to Eluate
- Evaporate to 100 μ L at ≤ 40 °C.

DERIVATIZATION:

Add 50 μ L HFBA (heptafluorobutyric anhydride). React 20 minutes at room temperature.

3 ANALYSIS

Inject 1 to 3 μ L of sample into chromatograph. Monitor the following ions:

Amphetamine	<i>d</i> ₅ -Amphetamine
240**	245**
91	91
118	123

Methamphetamine	<i>d</i> ₅ -Methamphetamine
254**	259**
210	210
118	123

*Suggested internal standards for GC/MS: *d*₅-Amphetamine and *d*₅-Methamphetamine.

Suggested internal standards for GC/FID: phentermine, propylamphetamine, other amphetamine analogs.

**Quantitation ion

4 OTHER EXTRACTION INFORMATION

Free bases of amphetamines are volatile. An alternative to the evaporation procedure above is to add 50 μ L methanolic HCl (MeOH:concentrated HCl; 9:1; v/v) to the eluent before evaporation. This forms the corresponding (nonvolatile) hydrochloride salts of the drugs.

ANALYTE NAME/MATRIX

M2707B

Amphetamines in Urine

ANALYTICAL TECHNIQUE

LC or LC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both nonpolar and cation-exchange mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 2 mL of urine, add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 0.1 M phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 1 mL 1.0 M acetic acid; draw through under vacuum
- Dry column for 5 minutes under vacuum
- 2 mL CH₃OH; draw through under vacuum
- Dry column (2 minutes at ≥ 10 inches Hg)

4. Elution

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily

5. Concentrate Eluate

- Add 100 μ L silylation grade DMF to eluate
- Evaporate to 100 μ L at ≤ 40 °C.

RECONSTITUTION:

Reconstitute the evaporated samples into 0.5 mL mobile phase (15% MeOH, 85% H₂O, 0.1% formic acid)

3 ANALYSIS

Inject 2 μ L of sample into the HPLC system, and monitor using triple quadrupole LC/MS/MS

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor Voltage (V)	Collision energy (V)
Amphetamine	136.1	119.1**	64	4
Amphetamine	136.1	91.1	64	14
Amphetamine-D ₆ *	142.1	125.1**	66	5
Amphetamine-D ₆ *	142.1	93.1**	66	13
MDA	180.1	163.1**	92	5
MDA	180.1	105.1	92	17
MDA-D ₅ *	185.1	168.1**	68	5
MDA-D ₅ *	185.1	110.1	68	21
MDEA	208.1	163.1**	88	8
MDEA	208.1	133.1	88	17
MDEA	208.1	105.1	88	21
MDEA-D ₆ *	214.2	166.1**	90	8
MDEA-D ₆ *	214.2	108.1	90	25
MDMA	194.1	163.1**	84	5
MDMA	194.1	135.1	84	17
MDMA	194.1	105.1	84	21
MDMA-D ₅ *	199.1	165.1**	82	4
MDMA-D ₅ *	199.1	107.1	82	25
Methamphetamine	150.1	119.1**	80	4
Methamphetamine	150.1	91.1	80	16
Methamphetamine-D ₉ *	159.2	125.2**	77	5
Methamphetamine-D ₉ *	159.2	93.1	77	13

MRM settings for Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source, operated in positive ESI mode.

*Suggested internal standards for LC/MS

** Quantitation ion

4 OTHER EXTRACTION INFORMATION

Free bases of amphetamines are volatile. An alternative to the evaporation procedure above is to add 50 μ L methanolic HCl (MeOH:conc. HCl; 9:1; v/v) to the eluent before evaporation. This forms the corresponding (nonvolatile) hydrochloride salts of the drugs.

ANALYTE NAME/MATRIX

M2708

Anabolic Steroids in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

β -GLUCURONIDASE HYDROLYSIS: To 5 mL of urine, add internal standard(s) and 2 mL of β -glucuronidase (5,000 F units/mL *Patella Vulgata* in 1.0 M acetate buffer, pH 5.0). Mix/vortex. Hydrolyze for 3 hours at 65 °C. Cool before proceeding. Adjust sample pH to 6.0 ± 0.5 with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 3 mL CH₃OH
- 3 mL DI H₂O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL 10% (v/v) CH₃OH in DI H₂O; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)
- 1 mL hexane or hexane/ethyl acetate (50/50); draw through under vacuum

4. Elution (choose a, b, c, or d)

- 3 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

- 3 mL CH₂Cl₂/IPA (80/20)
- 3 mL ethyl acetate
- 3 mL CH₃OH

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C.

DERIVATIZATION:

Add 50 μ L MSTFA (with 3% trimethylsilyliodide). Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate MSTFA solution.

3 ANALYSIS

Inject 1 to 3 μ L of sample (in MSTFA solution) into GC.

Principle Ions (Mass Selective Detection):

Testosterone-TMS	432, 301, 209
19-Noretiocholanone-TMS	405, 315, 225
Oxymetholone	640, 552, 462, 370, 143
Dehydroepiandrosterone-2TMS	432, 327, 297, 169
10-Nortestosterone-2TMS	418, 287, 194
Oxymetholone metab. #1	640, 552, 462, 143
Oxymetholone metab. #2	625, 462, 370, 143
11-B-Hydroxyandrosterone	522, 417, 158
Methandienone	409, 313, 281
19-Norandrosterone-2TMS	405, 315, 225
16-A-Hydroxyetiocholanone-TMS	504, 417
17-A-Epitestosterone-TMS	432, 341, 327, 209
Stanozolol-TMS	472, 381, 342, 149

ANALYTE NAME/MATRIX

M2709 Barbiturates in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

130 mg Bond Elut Certify
12102051 or 12113050

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3 ANALYSIS

Inject 1 to 2 μ L of sample into chromatograph. Monitor the following ions (Mass Selective Detection):

Amobarbital	Butobarbital	Butalbital	Hexobarbital	Pentobarbital	Phenobarbital	Secobarbital
156**	156**	168**	221**	156**	204**	168**
141	141	153	157	141	117	153
157	157	141	156	157	232	195

*Suggested internal standard for GC/MS: Hexobarbital

**Quantitation ion

3. Column Wash

- 1 mL 100 mM phosphate buffer (pH 6.0)/ CH₃OH (80/20); draw through under vacuum
- Dry column for 5 minutes under vacuum (≥ 10 inches Hg)
- 1 mL 1.0 M acetic acid; draw through under vacuum
- Dry column for 10 minutes under vacuum
- 1 mL hexane; draw through under vacuum
- Dry column for 2 minutes under vacuum

4. Elution

4 mL hexane/ethyl acetate (75/25); collect eluate at ≤ 5 mL/minute

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C. Reconstitute with 100 μ L ethyl acetate.

ANALYTE NAME/MATRIX

M2710
Basic Drugs from Urine

ANALYTICAL TECHNIQUE

HPLC

PRODUCT/PART NUMBER

130 mg Bond Elut Certify
12102051 or 12113050

1 PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both nonpolar and cation-exchange mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 2 mL of urine, add internal standard(s) and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL DI H_2O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 2 mL DI H_2O ; draw through under vacuum
- 2 mL 100 mM HCl; draw through under vacuum
- 3 mL CH_3OH ; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{CH}_3\text{OH} / \text{NH}_4\text{OH}$ (98/2); collect eluate at 1 to 2 mL/minute

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

3 ANALYSIS

Reconstitute in mobile phase and inject into chromatograph.

ANALYTE NAME/MATRIX

M2711 Benzodiazepines in Serum or Plasma

ANALYTICAL TECHNIQUE

HPLC

PRODUCT/PART NUMBER

130 mg Bond Elut Certify
12102051 or 12113050

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 1 mL serum or plasma, add internal standard and 1.0 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL DI H_2O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H_2O ; draw through under vacuum
- 1 mL 100 mM HCl or 1.0 M acetic acid; draw through under vacuum
- 3 mL CH_3OH ; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{CH}_2\text{Cl}_2/\text{IPA}/\text{NH}_4\text{OH}$ (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

3 ANALYSIS

Reconstitute in mobile phase and inject into chromatograph.

ANALYTE NAME/MATRIX

M2712 Benzodiazepines in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

130 mg Bond Elut Certify
12102051 or 12113050

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences. This method uses enzymatic hydrolysis for removal of conjugates prior to extraction.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

β -GLUCURONIDASE HYDROLYSIS: To 5 mL of urine, add internal standard(s)* and 2 mL of β -glucuronidase (5,000 F units/mL Patella Vulgata in 1.0 M acetate buffer, pH 5.0). Mix/vortex. Hydrolyze for 3 hours at 65 °C. Cool before proceeding.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL DI H₂O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 2 mL DI H₂O; draw through under vacuum
- 2 mL 20% acetonitrile in 100 mM phosphate buffer (pH 6.0); draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)
- 2 mL hexane; draw through under vacuum

4. Elution

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

3 ANALYSIS

Inject 1 to 3 μ L of sample (in BSTFA solution) into chromatograph. Principle Ions (Mass Selective Detection):

Alprazolam	308**, 279, 204
Clonazepam	387**, 352, 306
Desalkylflurazepam (TMS)	359**, 341, 245
Diazepam	256**, 283, 221
Halazepam	324**, 352, 289
Lorazepam (TMS)	429**, 430, 347
Nordiazepam (TMS)	341**, 342, 343
Oxazepam (TMS)	429**, 430, 313
Temazepam (TMS)	343**, 283, 257
Chlordiazepoxide	282**, 283, 284
α -Hydroxytriazolam	415*, 17, 430
α -Hydroxyalprazolam	381**, 396, 383
Hydroxyethylflurazepam	288**, 287, 289
Triazolam	313**, 314, 342
Prazepam	269**, 241, 324
4-Hydroxydiazepam	86**, 109, 307

*Suggested internal standard for GC/MS: Prazepam, *d*₅-Oxazepam

**Quantitation ion

4 OTHER INFORMATION

NOTE: Flurazepam does not extract under these conditions; however, metabolites such as desalkylflurazepam and hydroxyethyl-flurazepam will extract with high recovery. A basic wash is necessary in order to recover flurazepam; however, this reduces the recovery of other benzodiazepines.

ANALYTE NAME/MATRIX

M2713

Cocaine and Benzoyllecgonine in Serum, Plasma, or Whole Blood

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both nonpolar and cation-exchange mechanisms. At neutral pH, benzoyllecgonine (BE) carries both a positive and a negative charge; therefore, acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation-exchange at the amine functional group.

2 EXTRACTION METHOD

SAMPLE PROCEDURE:

Target analytes show poor hydrolytic stability, particularly under alkaline conditions. Samples should be kept cool and dark as much as possible after collection. Blood samples are best preserved with fluoride and kept at a pH of 5.

SAMPLE PREPARATION:

A. Serum or Plasma: To 1 mL of serum or plasma, add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B. Whole Blood: See Appendix A for sample treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 6 mL DI H₂O; draw through under vacuum
- 3 mL 1 M acetic acid; draw through under vacuum
- Dry column for 5 minutes under vacuum
- 6 mL CH₃OH; draw through under vacuum

4. Elution

2 mL CH₂Cl₂/IPA (80:20) containing 2% NH₄OH; collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.
PFPA derivatization is also acceptable.

3 ANALYSIS

Inject 1 to 3 μ L of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

Cocaine	<i>d</i> ₃ -Cocaine	TMS-BE	TMS- <i>d</i> ₃ -BE
182**	185**	240**	243**
198	201	256	259
303	306	361	364

*Suggested internal standards for GC/MS: *d*₃-Cocaine, *d*₃-Benzoyllecgonine.
Suggested internal standards for GC/FID: a) analogues of benzoyllecgonine (propylbenzoyllecgonine), b) opiate alkaloids (levallorphan, nalorphine, ethylmorphine, codeine), c) misc. (n-tetracosane, tetraphenylethylene (FID only), butylanthraquinone)

**Quantitation ion

ANALYTE NAME/MATRIX

M2714

Cocaine and Benzoylcegonine in Serum, Plasma, or Whole Blood

ANALYTICAL TECHNIQUE

HPLC

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both nonpolar and cation-exchange mechanisms. At neutral pH, benzoylcegonine (BE) carries both a positive and a negative charge; therefore, acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation-exchange at the amine functional group.

2 EXTRACTION METHOD

SAMPLE PROCEDURE:

Target analytes show poor hydrolytic stability, particularly under alkaline conditions. Samples should be kept cool and dark as much as possible after collection. Blood samples are best preserved with fluoride and kept at a pH of 5.

SAMPLE PREPARATION:

A. Serum or Plasma: To 1 mL of serum or plasma add internal standard(s) and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B. Whole Blood: See Appendix A for sample treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 6 mL DI H_2O ; draw through under vacuum
- 3 mL 1 M acetic acid; draw through under vacuum
- Dry for 5 minutes under vacuum
- 6 mL CH_3OH ; draw through under vacuum

4. Elution

2 mL $\text{CH}_2\text{Cl}_2/\text{IPA}/\text{NH}_4\text{OH}$ (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Concentrate

Evaporate to dryness at ≤ 40 °C

3 ANALYSIS

Reconstitute with 100 μL methanol and inject 20 μL onto the HPLC system.

ANALYTE NAME/MATRIX

M2715A

Cocaine and Benzoyllecgonine in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both nonpolar and cation-exchange mechanisms. At neutral pH, benzoyllecgonine (BE) carries both a positive and a negative charge; therefore, acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation-exchange at the amine functional group.

2 EXTRACTION METHOD

SAMPLE PROCEDURE:

Target analytes show poor hydrolytic stability, particularly under alkaline conditions. Samples should be kept cool and dark as much as possible after collection. Adjust sample to a pH of 5 with dilute acetic acid (0.1 M).

SAMPLE PREPARATION:

To 2 mL of urine, add internal standard(s) and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 6 mL DI H₂O; draw through under vacuum
- 3 mL 1 M acetic acid; draw through under vacuum
- Dry column for 5 minutes under vacuum
- 6 mL CH₃OH; draw through under vacuum

4. Elution

2 mL CH₂Cl₂/IPA (80:20) containing 2% NH₄OH; collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.
PFPA derivatization is also acceptable.

3 ANALYSIS

Inject 1 to 3 μ L of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

Cocaine	<i>d</i> ₃ -Cocaine	TMS-BE	TMS- <i>d</i> ₃ -BE
182**	185**	240**	243**
198	201	256	259
303	306	361	364

*Suggested internal standards for GC/MS: *d*₃-Cocaine, *d*₃-Benzoyllecgonine. Suggested internal standards for GC/FID: a) analogues of benzoyllecgonine (propylbenzoyllecgonine), b) opiate alkaloids (levallorphan, nalorphine, ethylmorphine, codeine), c) misc. (n-tetracosane, tetraphenylethylene (FID only), butylanthraquinone)

**Quantitation ion

ANALYTE NAME/MATRIX

M2715B Benzoylecgonine in Urine

ANALYTICAL TECHNIQUE

LC or LC/MS

PRODUCT/PART NUMBER

130 mg Bond Elut Certify 12102051 or 12113050

1 PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both nonpolar and cation-exchange mechanisms. At neutral pH, benzoylecgonine (BE) carries both a positive and a negative charge; therefore, acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation-exchange at the amine functional group.

2 EXTRACTION METHOD

SAMPLE PROCEDURE:

Target analytes show poor hydrolytic stability, particularly under alkaline conditions. Samples should be kept cool and dark as much as possible after collection. Adjust sample to a pH of 5 with dilute acetic acid (0.1 M).

SAMPLE PREPARATION:

To 2 mL of urine, add internal standard(s) and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

3 ANALYSIS

Inject 2 μ L of sample into the HPLC system, and monitor using triple quadrupole LC-MS/MS

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor Voltage (V)	Collision energy (V)
Benzoylecgonine (BE)	290.1	168.1**	90	15
BE	290.1	105.1	90	30
BE	290.1	82.1	90	32
d_8 -BE*	298.2	171.1**	90	15
d_8 -BE*	298.2	90	90	30

MRM settings for Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source, operated in positive ESI mode.

*Suggested internal standard for LC/MS: d_8 -Benzoylecgonine

** Quantitation ion

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 6 mL DI H_2O ; draw through under vacuum
- 3 mL 1 M acetic acid; draw through under vacuum
- Dry column for 5 minutes under vacuum
- 6 mL CH_3OH ; draw through under vacuum

4. Elution

2 mL $\text{CH}_2\text{Cl}_2/\text{IPA}$ (80:20) containing 2% NH_4OH ; collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

RECONSTITUTION:

Reconstitute dried samples into appropriate volume of mobile phase (10% methanol, 90% water, 0.1% formic acid).

ANALYTE NAME/MATRIX

M2716

Cocaine and Metabolites from Meconium

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both nonpolar and cation-exchange mechanisms. At neutral pH, benzoylecgonine (BE) carries both a positive and a negative charge; therefore, acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation-exchange at the amine functional group.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

Vortex 0.5 - 1 g meconium and 2 mL of CH₃OH. Centrifuge and transfer the supernatant to a clean tube. To each tube add 3 mL 100 mM phosphate buffer (pH 6.0), internal standard* and vortex. Matrix must be more aqueous than organic for good retention to occur.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 6 mL DI H₂O; draw through under vacuum
- 3 mL 1 M acetic acid; draw through under vacuum
- Dry column for 5 minutes under vacuum
- 6 mL CH₃OH; draw through under vacuum

4. Elution

2 mL CH₂Cl₂/IPA (80:20) containing 2% NH₄OH; collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Evaporate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

3 ANALYSIS

Inject 1 to 3 μ L of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

Cocaine	<i>d</i> ₃ -Cocaine	TMS-BE	TMS- <i>d</i> ₃ -BE
182**	185**	240**	243**
198	201	256	259
303	306	361	364

*Suggested internal standards for GC/MS: *d*₃-Cocaine, *d*₃-Benzoylecgonine.

Suggested internal standards for GC/FID: a) analogues of benzoylecgonine (propylbenzoylecgonine), b) opiate alkaloids (levallorphan, nalorphine, ethylmorphine, codeine), c) misc. (n-tetracosane, tetraphenylethylene (FID only), butylantraquinone)

**Quantitation ion

ANALYTE NAME/MATRIX

M2717

Cocaine and Metabolites from Meconium

ANALYTICAL TECHNIQUE

HPLC

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both nonpolar and cation-exchange mechanisms. At neutral pH, benzoylecgonine carries both a positive and a negative charge; therefore, acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation-exchange at the amine functional group.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

Vortex 0.5 - 1 g meconium and 2 mL of CH₃OH. Centrifuge and transfer the supernatant to a clean tube. To each tube add 3 mL 100 mM phosphate buffer (pH 6.0), internal standard* and vortex. Matrix must be more aqueous than organic for good retention to occur.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 6 mL DI H₂O; draw through under vacuum
- 3 mL 1 M acetic acid; draw through under vacuum
- Dry column for 5 minutes under vacuum
- 6 mL CH₃OH; draw through under vacuum

4. Elution

2 mL CH₂Cl₂/IPA (80:20) containing 2% NH₄OH; collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Evaporate

Evaporate to dryness at ≤ 40 °C

3 ANALYSIS

Reconstitute with 100 μ L methanol and inject 20 μ L onto the HPLC system.

*Suggested internal standard: Bupivacaine

ANALYTE NAME/MATRIX

M2718

Fentanyl and Analogues In Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences.

2 EXTRACTION METHOD

SAMPLE PROCEDURE:

To 2 mL of sample, add internal standard and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL DI H₂O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H₂O; draw through under vacuum
- 2 mL 1.0 M acetic acid; draw through under vacuum
- 3 mL CH₃OH; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Concentrate

Evaporate to dryness at ≤ 40 °C

3 ANALYSIS

Reconstitute with 50 μ L ethyl acetate. Inject 1 to 3 μ L of sample into chromatograph. Monitor the following ions (Mass Selective Detection):

Fentanyl	d ₅ -Fentanyl	α -Methylfentanyl
245*	250*	259*
146	151	203
189	194	146
p-Fluorofentanyl	3-Methylfentanyl	Thienfentanyl
263*	259*	245*
164	160	146
207	203	189
Sufentanil	Carfentanil	Lofentanil
289*	303*	317*
140	187	201
		289
Alfentanil		
289*		
268		
222		

*Quantitation ion

ANALYTE NAME/MATRIX

M2719
Flunitrazepam in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

130 mg Bond Elut Certify
12102051 or 12113050

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions to retain the drug and ion-exchange and secondary polar interactions for sample cleanup.

2 EXTRACTION METHOD

SAMPLE PROCEDURE:

To 2 mL of urine, add internal standard(s) and 2 mL of 0.1M phosphate buffer (adjusted to pH 6.0).

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- a) 2 mL CH₃OH
- b) 2 mL 0.1 M phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- a) 3 mL H₂O; draw through under vacuum
- b) 1 mL 1 M CH₃COOH; draw through under vacuum
- c) Dry 15 minutes at full vacuum
- d) 2 mL CH₃OH; draw through under vacuum

4. Elution

2 mL 2% NH₄OH in CH₂Cl₂:IPA (8:2)

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate under N₂ to dryness

3 ANALYSIS

Reconstitute in 50-100 μ L EtOAc. Inject into GC.

ANALYTE NAME/MATRIX

M2720

Fluoxetine and Norfluoxetine in Serum, Plasma, or Whole Blood

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both nonpolar and cation-exchange mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma: To 1 mL of serum or plasma, add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B. Whole Blood: See Appendix A for sample treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL DI H_2O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 2 mL CH_3OH ; draw through under vacuum
- 2 mL acetonitrile; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)
- 2 mL hexane/ethyl acetate (50:50, v/v); draw through under vacuum.

4. Elution

2 mL $\text{CH}_2\text{Cl}_2/\text{IPA}/\text{NH}_4\text{OH}$ (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Evaporate to 1 mL. Add 1 drop 0.3 N HCl in CH_3OH and vortex. Evaporate to dryness at room temperature under N_2 . Add 100 μL 1% Et_3N in toluene; vortex. Add 20 μL PFPA. React for 30 minutes at 90 °C. Allow to cool to ambient temperature. Evaporate to dryness at ambient temperature. Reconstitute in 100 μL hexane.

3 ANALYSIS

Reconstitute with 200 μL ethyl acetate. Inject 2 μL of sample into chromatograph. Monitor the following ions (Mass Selective Detection):

Norfluoxetine	Fluoxetine	Protriptyline
117**	117	191**
176	190**	409
280	294	

*Suggested internal standard: Protriptyline

**Quantitation ion

ANALYTE NAME/MATRIX

M2721

General Drug Screen from Urine or Plasma

ANALYTICAL TECHNIQUE

GC or GC/FID

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic, acidic, and neutral drugs can be retained and selectively eluted using the ion-exchange, polar, and nonpolar interactions of the Bond Elut Certify mixed mode sorbent.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 2 mL of either urine or plasma, add 2 mL of 0.1 M phosphate buffer (pH 6.0). Mix/vortex.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 0.1 M phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (2 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 1 mL DI H₂O; draw through under vacuum
- 0.5 mL 0.01 M acetic acid; draw through under vacuum
- Dry column (4 minutes at 15 inches Hg)
- 50 µL CH₃OH (no vacuum)
- Dry column (1 minute at 15 inches Hg)

4. Elution

- Acidic and neutral drugs (Fraction A)
4 mL acetone:chloroform (50/50); draw through slowly under low vacuum (1 inch Hg)
- Basic Drugs (Fraction B)
2 mL EtOAc/NH₄OH (98/2); use no vacuum

3 ANALYSIS

Add 100 µL of a 200 µg/mL Prazepam solution (internal standard). Mix/vortex. Evaporate each fraction to 100 µL at 40 °C under N₂. Inject 1 to 2 µL of each fraction into the GC.

4 OTHER INFORMATION

BOND ELUT CERTIFY GENERAL DRUG SCREEN ANALYSIS*

The following compounds have been extracted from urine and plasma samples using Bond Elut Certify extraction columns:

FRACTION (A): ACIDIC AND NEUTRAL DRUGS

Butalbital	Clonazepam	Methaqualone
Heptabarbital	Diazepam	Meprobamate
Hexobarbital	Lorazepam	
Metharbital	Nitrazepam	
Pentobarbital	Oxazepam	
Probarbital		
Secobarbital		

FRACTION (B): BASIC DRUGS

Amphetamine	Levallorphan	Procaine
Cocaine	Mepivacaine	Promethazine
Codeine	Methamphetamine	Trimipramine
Imipramine	Morphine**	

*Adapted from Chen, X.-H. et al. *Journal of Forensic Sciences* 1992, 37(1), 61-71.

**Requires 2 x 2 mL EtOAc/NH₄OH (98/2) elution aliquots.

ANALYTE NAME/MATRIX

M2722

Lysergic Acid Diethylamide (LSD) in Serum, Plasma, or Whole Blood

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using nonpolar and cation-exchange mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma: To 1 mL of serum or plasma, add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B. Whole Blood: See Appendix A for sample treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL DI H_2O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H_2O ; draw through under vacuum
- 1 mL 1.0 M acetic acid; draw through under vacuum
- 3 mL CH_3OH ; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{CH}_2\text{Cl}_2/\text{IPA}/\text{NH}_4\text{OH}$ (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Add 20 μL acetonitrile and 20 μL BSTFA (with 1% TMCS). Blanket with N_2 and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

3 ANALYSIS

Inject 1 to 3 μL of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

LSD	d_3 -LSD
395**	398**
293	296
268	271

*Suggested internal standard for GC/MS: d_3 -LSD

**Quantitation ion

ANALYTE NAME/MATRIX

M2723

**Lysergic Acid Diethylamide (LSD)
in Urine**

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using nonpolar and cation-exchange mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine, add internal standard* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL DI H₂O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H₂O; draw through under vacuum
- 1 mL 1.0 M acetic acid; draw through under vacuum
- 3 mL CH₃OH; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Add 20 μ L acetonitrile and 20 μ L BSTFA (with 1% TMCS). Blanket with N₂ and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

3 ANALYSIS

Inject 1 to 3 μ L of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

LSD	<i>d</i> ₃ -LSD
395**	398**
293	296
268	271

*Suggested internal standard for GC/MS: *d*₃-LSD

**Quantitation ion

ANALYTE NAME/MATRIX

M2724 Meperidine (Pethidine) in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

130 mg Bond Elut Certify
12102051 or 12113050

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using nonpolar and cation-exchange mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine, add internal standard* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL DI H₂O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H₂O; draw through under vacuum
- 1 mL 1.0 M acetic acid; draw through under vacuum
- 3 mL CH₃OH; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C. Remove immediately upon completion. Reconstitute with 100 μ L ethyl acetate.

3 ANALYSIS

Inject 1 to 3 μ L of sample into chromatograph. Monitor the following ions (Mass Selective Detection):

Meperidine	Phenyltoloxamine
247**	58**
218	
172	

*Suggested internal standard for GC/MS: Phenyltoloxamine

**Quantitation ion

ANALYTE NAME/MATRIX

M2725
Methadone in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

130 mg Bond Elut Certify
12102051 or 12113050

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using nonpolar and cation-exchange mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine, add internal standard* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL DI H_2O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H_2O ; draw through under vacuum
- 1 mL 1.0 M acetic acid; draw through under vacuum
- 3 mL CH_3OH ; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL 2% NH_4OH in ethyl acetate; collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Concentrate

Evaporate to dryness at ≤ 40 °C. Reconstitute with 100 μL ethyl acetate.

3 ANALYSIS

Inject 1 to 3 μL of sample into chromatograph.
Monitor the following ions (Mass Selective Detection):

Methadone
72**
91
165

*Suggested internal standard for GC/MS: d_3 -Methadone

**Quantitation ion

ANALYTE NAME/MATRIX

M2726

Methaqualone in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine, add internal standard* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL DI H₂O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H₂O; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)
- 2 mL hexane; draw through under vacuum

4. Elution

2 mL hexane/ethyl acetate (50/50); collect eluate at ≤ 5 mL/minute

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C. Reconstitute with 50 μ L ethyl acetate.

3 ANALYSIS

Inject 1 to 3 μ L of sample into chromatograph. Monitor the following ions (Mass Selective Detection):

Methaqualone	Hexobarbital
235**	221**
250	157
233	156

*Suggested internal standard for GC/MS: Hexobarbital

**Quantitation ion

ANALYTE NAME/MATRIX

M2727A

6-Monoacetyl Morphine in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using cation-exchange and nonpolar mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine, add internal standard* and 2 mL of 10 mM phosphate buffer (pH 6.0). Mix/vortex. Adjust pH to 8.0-8.5 with KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 10 mM phosphate buffer (adjusted to pH 8.0-9.0 with 10 M KOH)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 2 mL DI H₂O; draw through under vacuum
- 2 mL 10 mM phosphate buffer (adjusted to pH 4.0 with phosphoric acid); draw through under vacuum
- 2 mL CH₃OH; draw through under vacuum
- Dry column (2 minutes at ≥ 10 inches Hg)

4. Elution

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

3 ANALYSIS

Inject 1 to 3 μ L of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

TMS-6-MAM	TMS- <i>d</i> ₃ -6-MAM
399**	402**
340	343
287	290

*Suggested internal standard for GC/MS: *d*₃-6-MAM

**Quantitation ion

ANALYTE NAME/MATRIX

M2727B

6-Monoacetyl Morphine in Urine

ANALYTICAL TECHNIQUE

LC or LC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using cation-exchange and nonpolar mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine, add internal standard* and 2 mL of 10 mM phosphate buffer (pH 6.0). Mix/vortex. Adjust pH to 8.0-8.5 with KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 10 mM phosphate buffer (adjusted to pH 8.0-9.0 with 10 M KOH)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

3 ANALYSIS

Inject 2 μ L of sample into the HPLC system, and monitor using triple quadrupole LC/MS/MS.

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor Voltage (V)	Collision energy (V)
6-acetylmorphine (6-AM)	328.2	165.1**	140	40
6-AM	328.2	211.1	140	25
6-AM	328.2	193.1	140	25
d ₆ -6AM**	334.2	165.1**	140	40
d ₆ -6AM**	334.2	211.1	140	25

MRM settings for Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source, operated in positive ESI mode

*Suggested internal standard for LC/MS: d₆-6AM

** Quantitation ion

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 2 mL DI H₂O; draw through under vacuum
- 2 mL 10 mM phosphate buffer (adjusted to pH 4.0 with phosphoric acid); draw through under vacuum
- 2 mL CH₃OH; draw through under vacuum
- Dry column (2 minutes at ≥ 10 inches Hg)

4. Elution

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C. Reconstitute in initial mobile phase (10% methanol, 90% water, 0.1% formic acid).

ANALYTE NAME/MATRIX

M2728
Nicotine in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

130 mg Bond Elut Certify
12102051 or 12113050

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using cation-exchange and nonpolar mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 2 mL of urine, add internal standard(s) and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 1 mL 1.0 M acetic acid; draw through under vacuum
- Dry column for 5 minutes under vacuum
- 6 mL CH_3OH ; draw through under vacuum
- Dry column (2 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{CH}_2\text{Cl}_2/\text{IPA}/\text{NH}_4\text{OH}$ (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C under N_2 . Remove immediately upon completion. Reconstitute with 50 μL ethyl acetate.

ANALYTE NAME/MATRIX

M2729 Opiates (Free/Unbound) in Serum, Plasma, or Whole Blood

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using cation-exchange and nonpolar mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma: To 1 mL of serum or plasma, add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Adjust pH to 8.0-8.5 with 10 M KOH.

B. Whole Blood: See Appendix A for sample treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Adjust pH to 8.0-8.5 with 10 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 100 mM phosphate buffer (adjusting to pH 8.0-9.0 with 10 M KOH)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 2 mL DI H₂O; draw through under vacuum
- 2 mL 100 mM acetate buffer (pH 4.0); draw through under vacuum
- 2 mL CH₃OH; draw through under vacuum
- Dry column (2 minutes at ≥ 10 inches Hg)

4. Elution

2 mL CH₃OH/NH₄OH (98/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C.

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

3 ANALYSIS

Inject 1 to 2 μ L of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

TMS-Codeine	TMS-d ₃ -Codeine	TMS-Morphine	TMS-d ₃ -Morphine
371**	374**	429**	432**
234	237	287	290
343	346	324	337

*Suggested internal standard for GC/MS: d₃-Codeine, d₃-Morphine.

Suggested internal standards for other GC: Nalorphine, Alkanes, such as Tetracosane or Docosane

**Quantitation ion

ANALYTE NAME/MATRIX

M2730A Opiates in Urine

ANALYTICAL TECHNIQUE

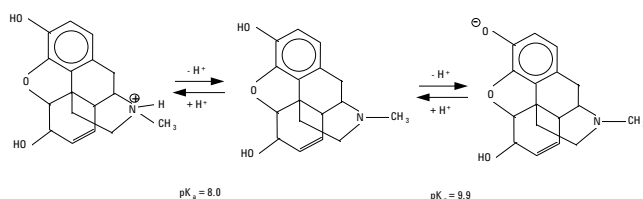
GC or GC/MS

PRODUCT/PART NUMBER

130 mg Bond Elut Certify
12102051 OR 12113050

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using cation-exchange and nonpolar mechanisms. Morphine can behave as either an acid or a base depending on the sample pH. Therefore, careful monitoring of pH in sample preparation is crucial for reproducible recoveries (See also section 4).



2 EXTRACTION METHOD

SAMPLE PREPARATION: (choose A or B)

A. Enzymatic Hydrolysis of Glucuronide: To 2 mL of urine, add internal standard(s)* and 2 mL of β -glucuronidase: 5,000 F units/mL Patella Vulgata in 1.0 M acetate buffer (pH 5.0). Mix/vortex. Hydrolyze for 3 hours at 65 °C. Cool before proceeding. Adjust sample pH to 8.0-8.5 with 10 M KOH.

B. Acid Hydrolysis of Glucuronide (see also section 4): To 2 mL of urine, add internal standard(s)* and 1 mL concentrated HCl. Mix/vortex. Immerse in a hot water bath for at least 30 minutes at 100 °C. Cool before proceeding. Add 2 mL 0.1 M potassium phosphate buffer, pH 6. Mix/vortex. Adjust sample pH to between 8.0-8.5 with 10 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL 100 mM phosphate buffer (adjusted to pH 8.0-9.0 with 10 M KOH)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 2 mL DI H_2O ; draw through under vacuum
- 2 mL 100 mM acetate buffer (pH 4.0); draw through under vacuum
- 2 mL CH_3OH ; draw through under vacuum
- Dry column (2 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{CH}_3\text{OH}/\text{NH}_4\text{OH}$ (98/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Add 50 μL BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

Other acceptable derivatization reagents: MBTFA, PFPA, TFAA

3 ANALYSIS

Inject 1 to 2 μL of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

TMS-Codeine	TMS- d_3 -Codeine	TMS-Morphine	TMS- d_3 -Morphine
371**	374**	429**	432**
234	237	287	290
343	346	324	337

*Suggested internal standard for GC/MS: d_3 -Codeine, d_3 -Morphine.

Suggested internal standards for other GC: nalorphine, alkanes, such as tetracosane or docosane.

**Quantitation ion

4 ADDITIONAL TIPS ON ACID HYDROLYSIS

Successful Bond Elut Certify extraction after acid hydrolysis relies heavily on careful pH adjustment up to pH 8.5. It is very important that the pH never exceeds 8.5. (If it does, significant decrease in morphine recovery with little change in codeine recovery will be observed.) An excellent way to achieve proper pH is to titrate the working solution of 10 M KOH on blank samples to determine the volume of KOH required to adjust 1 mL of concentrated HCl to a pH of 8.5:

- Add 2 mL DI water followed by 1.0 mL concentrated HCl to a 16 x 100 mm test tube
- Add 2 mL 1 M potassium phosphate buffer, pH 6
- Add 0.8 mL 10 M KOH and measure pH with a pH meter
- Add 100 μL 10 M KOH and measure pH
- Continue to add 100 mL aliquots of KOH until pH 8.5 is reached
- Note total volume used

ANALYTE NAME/MATRIX

M2730B Opiates in Urine

ANALYTICAL TECHNIQUE

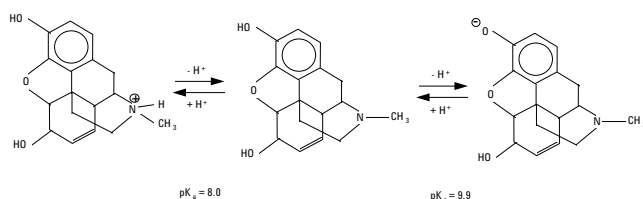
LC or LC/MS

PRODUCT/PART NUMBER

130 mg Bond Elut Certify 12102051 OR 12113050

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using cation-exchange and nonpolar mechanisms. Morphine can behave as either an acid or a base depending on the sample pH. Therefore, careful monitoring of pH in sample preparation is crucial for reproducible recoveries (See also section 4).



2 EXTRACTION METHOD

SAMPLE PREPARATION: (choose A or B)

A. Enzymatic Hydrolysis of Glucuronide: To 2 mL of urine, add internal standard(s)* and 2 mL of β -glucuronidase: 5,000 F units/mL Patella Vulgata in 1.0 M acetate buffer (pH 5.0). Mix/vortex. Hydrolyze for 3 hours at 65 °C. Cool before proceeding. Adjust sample pH to 8.0-8.5 with 10 M KOH.

B. Acid Hydrolysis Of Glucuronide (see also section 4): To 2 mL of urine, add internal standard(s)* and 1 mL concentrated HCl. Mix/vortex. Immerse in a hot water bath for at least 30 minutes at 100 °C. Cool before proceeding. Add 2 mL 0.1 M potassium phosphate buffer, pH 6. Mix/vortex. Adjust sample pH to between 8.0-8.5 with 10 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL 100 mM phosphate buffer (adjusted to pH 8.0-9.0 with 10 M KOH)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 2 mL DI H_2O ; draw through under vacuum
- 2 mL 100 mM acetate buffer (pH 4.0); draw through under vacuum
- 2 mL CH_3OH ; draw through under vacuum
- Dry column (2 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{CH}_3\text{OH} / \text{NH}_4\text{OH}$ (98/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C. Reconstitute in initial mobile phase (5% methanol, 95% water, 0.1% formic acid).

3 ANALYSIS

Inject 2 μ L of sample into the HPLC system, and monitor using triple quadrupole LC/MS/MS.

Compound	Precursor Ion (<i>m/z</i>)	Product Ion (<i>m/z</i>)	Fragmentor Voltage (V)	Collision energy (V)
Codeine	300.2	215.1**	130	23
Codeine	300.2	165.1	130	46
Codeine	300.2	153.1	130	50
<i>d</i> ₆ -Codeine*	306.2	165.1**	130	44
<i>d</i> ₆ -Codeine*	306.2	218.1	130	23
Morphine	286.1	201.1**	130	23
Morphine	286.1	181.1	130	40
Morphine	286.1	165.1	130	43
<i>d</i> ₆ -Morphine*	292.1	181.1**	130	40
<i>d</i> ₆ -Morphine*	292.1	165.1	130	42
Morphine-3-glucuronide	462.2	286.1**	162	45
Oxycodone	316.2	298.1**	130	15
Oxymorphone	302.2	284.1**	130	17
Hydrocodone	300.2	199.1**	130	30
Norcodeine	286.1	225.1**	130	20
Hydromorphone	286.1	185.1**	130	28

MRM settings for Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source, operated in positive ESI mode.

*Suggested internal standard for LC/MS: *d*₆-Codeine, *d*₆-Morphine

** Quantitation ion

4 ADDITIONAL TIPS ON ACID HYDROLYSIS

Successful Bond Elut Certify extraction after acid hydrolysis relies heavily on careful pH adjustment up to pH 8.5. It is very important that the pH never exceeds 8.5. (If it does, significant decrease in morphine recovery with little change in codeine recovery will be observed.) An excellent way to achieve proper pH is to titrate the working solution of 10 M KOH on blank samples to determine the volume of KOH required to adjust 1 mL of concentrated HCl to a pH of 8.5:

1. Add 2 mL DI water followed by 1.0 mL concentrated HCl to a 16 x 100 mm test tube
2. Add 2 mL 1 M potassium phosphate buffer, pH 6
3. Add 0.8 mL 10 M KOH and measure pH with a pH meter
4. Add 100 μ L 10 M KOH and measure pH
5. Continue to add 100 mL aliquots of KOH until pH 8.5 is reached
6. Note total volume used

ANALYTE NAME/MATRIX

M2731A

Phencyclidine (PCP) in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using cation-exchange and nonpolar mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 1 mL of urine, add internal standard* and 0.5 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 1 mL CH_3OH
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 1 mL 1.0 M acetic acid; draw through under vacuum
- 2 mL CH_3OH ; draw through under vacuum
- Dry column (2 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{ACN}/\text{NH}_4\text{OH}$ (98/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C under N_2 . Remove immediately upon completion. Reconstitute with 50 μL hexane.

3 ANALYSIS

Inject 1 to 2 μL of sample into chromatograph.

Monitor the following ions (Mass Selective Detection):

Phencyclidine	d_5 -Phencyclidine
200**	248**
186	246
242	

*Suggested internal standard for GC/MS: d_5 -Phencyclidine
Suggested internal standard for non-GC/MS: Ketamine

**Quantitation ion

ANALYTE NAME/MATRIX

M2731B

Phencyclidine (PCP) in Urine

ANALYTICAL TECHNIQUE

LC or LC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using cation-exchange and nonpolar mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 1 mL of urine, add internal standard* and 0.5 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 1 mL CH_3OH
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

3 ANALYSIS

Inject 2 μL of sample into the HPLC system, and monitor using triple quadrupole LC/MS/MS

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor Voltage (V)	Collision energy (V)
PCP	244.2	86.1**	80	7
PCP	244.2	159.1	80	7
PCP	244.2	91.1	80	34
d_5 -PCP*	249.2	164.1**	80	7
d_5 -PCP*	249.2	86.1	80	7

MRM settings for Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source, operated in positive ESI mode.

*Suggested internal standard for LC/MS: d_5 -PCP

** Quantitation ion

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 1 mL 1.0 M acetic acid; draw through under vacuum
- 2 mL CH_3OH ; draw through under vacuum
- Dry column (2 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{ACN}/\text{NH}_4\text{OH}$ (98:2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C under N_2 . Remove immediately upon completion. Reconstitute with 0.5 mL 30:70 $\text{ACN}:\text{H}_2\text{O}$ + 0.1% formic acid.

ANALYTE NAME/MATRIX

M2732

Propoxyphene in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using cation-exchange and nonpolar mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine, add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL DI H_2O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H_2O ; draw through under vacuum
- 1 mL 1.0 M acetic acid; draw through under vacuum
- 3 mL CH_3OH ; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{CH}_2\text{Cl}_2/\text{IPA}/\text{NH}_4\text{OH}$ (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Concentrate

Evaporate to dryness at ≤ 40 °C. Reconstitute with 100 μL ethyl acetate.

3 ANALYSIS

Inject 1 to 3 μL of sample into chromatograph.

Monitor the following ions (Mass Selective Detection):

Propoxyphene	Phenyltoloxamine
58**	58**
115	
208	

*Suggested internal standard for GC/MS: d_5 -Propoxyphene or Phenyltoloxamine

**Quantitation ion

ANALYTE NAME/MATRIX

M2733

Sertraline and Desmethylsertraline in Serum, Plasma, or Whole Blood

ANALYTICAL TECHNIQUE

HPLC

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Basic drug extraction using cation-exchange and nonpolar mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma: To 1 mL of serum or plasma, add internal standard(s) and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B: Whole Blood: See Appendix A for sample treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL DI H₂O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H₂O; draw through under vacuum
- 1 mL 1.0 M acetic acid; draw through under vacuum
- 3 mL CH₃OH; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

3 ANALYSIS

Reconstitute with 200 μ L acetonitrile/DI H₂O (25/75). Mix/vortex vigorously for 30 seconds. Inject 100 μ L into chromatograph at wavelength 235 nm. Mobile phase (from literature) = 0.25 M K₂HPO₄ (pH 2.7) containing 30% CH₃CN. Flow rate 2 mL/minute.

ANALYTE NAME/MATRIX

M2734

**THC and Carboxy-THC
in Serum, Plasma, or Whole Blood**

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences.

2 EXTRACTION METHOD

SAMPLE PREPARATION: (Choose A, B, or C)

Serum, Plasma, or Whole Blood:

- To 1 mL of sample, add internal standard(s)* and 1 mL acetonitrile. Mix/vortex. Centrifuge and transfer supernatant to a clean test tube. To the supernatant, add 5 mL 100 mM acetic acid.
- To 1 mL sample, add internal standard(s)* and 2 mL 30% acetonitrile. Mix/vortex. Centrifuge and transfer supernatant to a clean test tube. To the supernatant, add 5 mL 100 mM acetate buffer (pH 4.0).

Serum or Plasma:

- To 1 mL sample, add internal standard(s)* and 5 mL 100 mM acetate buffer (pH 4.0). Mix/vortex and centrifuge.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 50 mM phosphoric acid

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 9 mL 50 mM phosphoric acid; draw through under vacuum
- 3 mL 50 mM phosphoric acid/CH₃OH (80/20); draw through under vacuum
- Dry column (10 minutes at ≥ 10 inches Hg)
- 200 μ L hexane; draw through under vacuum

4. Elution

1 mL hexane/ethyl acetate (80/20); collect eluate at ≤ 5 mL/minute

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

Other acceptable derivatization reagents: BSA, MSTFA, MTBSTFA, PFBBR, TFAA, TMPAH, TMSI

3 ANALYSIS

Inject 1 to 3 μ L of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

THC	<i>d</i> ₃ -THC	Carboxy- Δ^9 -THC	<i>d</i> ₃ -Carboxy Δ^9 -THC
303**	306**	371**	374**
315	318	473	476
386	389	488	491

*Suggested internal standards for GC/MS: *d*₃-THC and *d*₃-Carboxy- Δ^9 -THC

Suggested internal standards for other GC: Cannabinol, Oxyphenbutazone, Ketoprofen

**Quantitation ion

ANALYTE NAME/MATRIX

M2735A

THC and Carboxy-THC in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

BASE HYDROLYSIS OF GLUCURONIDE: To 3 mL of urine, add internal standards* and 300 μ L of 10 M KOH. Mix/vortex. Hydrolyze for 15 minutes at 60 °C. Cool before proceeding. Add 400 μ L glacial acetic acid and 3 mL 50 mM phosphoric acid. Mix/vortex. Sample pH should be between 4.0-5.0. If not, adjust the pH to this range.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL 50 mM phosphoric acid

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL 50 mM phosphoric acid; draw through under vacuum
- 3 mL 50 mM phosphoric acid/ CH_3OH (80/20); draw through under vacuum
- Dry column (3 minutes at ≥ 10 inches Hg)
- 200 μ L hexane; draw through under vacuum

4. Elution

2 mL hexane/ethyl acetate (80/20); collect eluate at ≤ 5 mL/minute

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 90 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA.

Other acceptable derivatization reagents: BSA, MSTFA, MTBSTFA, PFBBr, TFAA, TMPAH, TMSI

3 ANALYSIS

Inject 1 to 3 μ L of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

THC	d_3 -THC	Carboxy- Δ^9 -THC	d_3 -Carboxy- Δ^9 -THC
303**	306**	371**	374**
315	318	473	476
386	389	488	491

*Suggested internal standards for GC/MS: d_3 -THC and d_3 -Carboxy- Δ^9 -THC

Suggested internal standards for other GC: Cannabinol, Oxyphenbutazone, Ketoprofen

**Quantitation ion

ANALYTE NAME/MATRIX

M2735B

THC and Carboxy-THC in Urine

ANALYTICAL TECHNIQUE

LC or LC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

BASE HYDROLYSIS OF GLUCURONIDE: To 3 mL of urine, add internal standards* and 300 μ L of 10 M KOH. Mix/vortex. Hydrolyze for 15 minutes at 60 °C. Cool before proceeding. Add 400 μ L glacial acetic acid and 3 mL 50 mM phosphoric acid. Mix/vortex. Sample pH should be between 4.0-5.0. If not, adjust the pH to this range.

3 ANALYSIS

Inject 10 μ L of sample into the HPLC system, and monitor using triple quadrupole LC/MS/MS

Compound	Precursor Ion (<i>m/z</i>)	Product Ion (<i>m/z</i>)	Fragmentor Voltage (V)	Collision energy (V)
11-nor-9-carboxy- Δ^9 -THC	343.2	299.2**	135	18
11-nor-9-carboxy- Δ^9 -THC	343.2	245.1	135	30
11-nor-9-carboxy- Δ^9 -THC	343.2	191.1	135	33
d_9 -11-nor-9-carboxy- Δ^9 -THC*	352.2	308.2**	145	18
d_9 -11-nor-9-carboxy- Δ^9 -THC*	352.2	254.2	145	30
11-nor-9-carboxy- Δ^9 -THC glucuronide	519.2	343.2**	160	22
11-nor-9-carboxy- Δ^9 -THC glucuronide	519.2	299.2	160	36

MRM settings for Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source, operated in negative ESI mode.

*Suggested internal standard for LC/MS: d_9 -11-nor-9-carboxy- Δ^9 -THC

** Quantitation ion

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 50 mM phosphoric acid

NOTE: Use a low vacuum (\leq 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL 50 mM phosphoric acid; draw through under vacuum
- 3 mL 50 mM phosphoric acid/CH₃OH (80/20); draw through under vacuum
- Dry column (3 minutes at \geq 10 inches Hg)
- 200 μ L hexane; draw through under vacuum

4. Elution

2 mL hexane/ethyl acetate (80/20); collect eluate at \leq 5 mL/minute

5. Dry Eluate

Evaporate to dryness at \leq 40 °C. Reconstitute in initial mobile phase (30% methanol, 70% 5 mM ammonium formate).

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 90 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA.

Other acceptable derivatization reagents: BSA, MSTFA, MTBSTFA, PFBBr, TFAA, TMPAH, TMSI

ANALYTE NAME/MATRIX

M2736

Tricyclic Antidepressants in Serum, Plasma, or Whole Blood

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma: To 1 mL of serum or plasma, add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B. Whole Blood: See Appendix A for sample treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL DI H_2O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H_2O ; draw through under vacuum
- 1 mL 1.0 M acetic acid; draw through under vacuum
- 3 mL CH_3OH ; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{CH}_2\text{Cl}_2/\text{IPA}/\text{NH}_4\text{OH}$ (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

DERIVATIZATION:

Reconstitute with 50 μL ethyl acetate. Add 50 μL of pentafluoropropionic anhydride (PFPA) to derivatize. Blanket with N_2 and cap. React 20 minutes at 70 °C. Evaporate to dryness at ≤ 40 °C. Reconstitute with 100 μL ethyl acetate.

NOTE: Underivatized analyte can also be analyzed.

3 ANALYSIS

A. UNDERIVATIZED ANALYSIS

Reconstitute with 100 μL CH_3OH . Inject 1-3 μL onto GC/NPD or GC/MS

B. DERIVATIZED (PFPA) ANALYSIS

Inject 1 to 3 μL onto GC/NPD or GC/MS

*Suggested internal standards: Clomipramine or Protriptyline

ANALYTE NAME/MATRIX

M2737

Tricyclic Antidepressants in Serum, Plasma, or Whole Blood

ANALYTICAL TECHNIQUE

HPLC

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify
12102051 or 12113050**

1 PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion-exchange and secondary polar interactions to remove interferences.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma: To 1 mL of serum or plasma, add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B. Whole Blood: See Appendix A for sample treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify Cartridge Conditioning

- 2 mL CH_3OH
- 2 mL DI H_2O
- 1 mL 100 mM phosphate buffer (pH 6.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 3 mL DI H_2O ; draw through under vacuum
- 1 mL 1.0 M acetic acid; draw through under vacuum
- 3 mL CH_3OH ; draw through under vacuum
- Dry column (5 minutes at ≥ 10 inches Hg)

4. Elution

2 mL $\text{CH}_2\text{Cl}_2/\text{IPA}/\text{NH}_4\text{OH}$ (78/20/2); collect eluate at 1 to 2 mL/minute

NOTE: Prepare elution solvent daily.

5. Dry Eluate

Evaporate to dryness at ≤ 40 °C

3 ANALYSIS

Reconstitute with 200 μL acetonitrile/DI H_2O (25/75). Mix/vortex vigorously for 30 seconds. Inject 100 μL into chromatograph.

*Suggested internal standards: Trimipramine and Protriptyline

BOND ELUT CERTIFY II EXTRACTION METHODS

INTRODUCTION AND OVERVIEW
OF THE MANUAL

SOLVENTS, SOLVENT MIXTURES,
REAGENTS, AND SOLUTIONS

EQUIPMENT AND ACCESSORIES

BOND ELUT CERTIFY
EXTRACTION METHODS

BOND ELUT CERTIFY II
EXTRACTION METHODS

APPENDIX

ANALYTE NAME/MATRIX

M2738

Barbiturates In Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify II
12102080 or 12113051**

1 PRINCIPLE AND MECHANISMS

Acidic drug extraction using a nonpolar mechanism for retention. Applicable drugs include amobarbital, butabarbital, pentobarbital, phenobarbital, secobarbital, and methaqualone.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

Add internal standard(s)* and 2 mL of 100 mM sodium acetate buffer (pH 7.0).

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify II Cartridge Conditioning

- 1 mL CH₃OH
- 1 mL 100 mM sodium acetate buffer (pH 7.0)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

3 ANALYSIS

Reconstitute in 100 μ L ethyl acetate. Inject 1 to 2 μ L of sample into chromatograph. Monitor the following ions (Mass Selective Detection):

Amobarbital	Butabarbital	Butalbital	Hexobarbital	Pentobarbital	Phenobarbital	Secobarbital
156**	156**	168**	221**	156**	204**	168**
141	141	153	157	141	117	153
157	157	141	156	157	232	195

*Suggested internal standard for GC/MS: Hexobarbital

**Quantitation ion

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 1 mL 100 mM sodium acetate buffer (pH 7.0); draw through under vacuum
- Dry for 5 minutes at full vacuum
- 2 mL hexane/ethyl acetate (95:5)

4. Elution

2 mL hexane/ethyl acetate (75:25); collect eluate at 1 to 2 mL/minute

5. Dry Extract

Evaporate solvent at room temperature under a slow stream of nitrogen.

ANALYTE NAME/MATRIX

M2739

**Non-Steroidal Anti-Inflammatory Drugs
in Urine**

ANALYTICAL TECHNIQUE

HPLC

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify II
12102080 or 12113051**

1 PRINCIPLE AND MECHANISMS

Acidic drug extraction using a nonpolar mechanism for retention. Applicable drugs include salicylic acid, naproxen, ibuprofen, indomethacin. If salicylic acid is not assayed, anion-exchange can also be employed as a retention mechanism.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

Hydrolyze 2 mL of urine with 200 μ L 10 M KOH for 15 minutes at 60 °C. Cool; adjust pH to 2.0 with concentrated HCl. Add internal standard(s) and 2 mL 10 mM sodium acetate buffer (pH 2.0).

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify II Cartridge Conditioning

- 2 mL CH₃OH
- 2 mL 10 mM sodium acetate buffer (pH 2.0)

NOTE: Use a low vacuum (\leq 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 2 mL 10 mM sodium acetate buffer (pH 2.0); draw through under vacuum
- 2 mL 10% aqueous acetic acid
- Dry 5 minutes at full vacuum

4. Elution

2 mL 100 mM phosphoric acid/acetonitrile (1:1); collect eluate at 1 to 2 mL/minute

3 ANALYSIS

Inject extract into the HPLC column for analysis.

ANALYTE NAME/MATRIX

M2740

11-Nor- Δ^9 -Tetrahydrocannabinol-9-Carboxylic Acid (THC Metabolite) in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**130 mg Bond Elut Certify II
12102080 or 12113051**

1 PRINCIPLE AND MECHANISMS

Acidic drug extraction using nonpolar and anion-exchange mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

Hydrolyze 3 mL of urine with 300 μ L 10 M KOH for 15 minutes at 60 °C. Cool; add 165 μ L glacial acetic acid; pH should be between 4.5-6.5. Add internal standard(s)* and 2 mL 0.1 M sodium acetate solution, adjusted to pH 7, and containing 5% (vol) methanol.

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify II Cartridge Conditioning

- 1 mL CH₃OH
- 1 mL 0.1 M sodium acetate solution, adjusted to pH 7 with HCl, and containing 5% (vol) methanol

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- 2 mL 50% methanol; draw through under vacuum
- Dry column 1 minute at full vacuum

IMPORTANT: Do not exceed drying time.

4. Elution

2 mL hexane/ethyl acetate (75:25) with 1% glacial acetic acid; collect eluate at 1 to 2 mL/minute

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 90 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA.

Other acceptable derivatization reagents: BSA, MSTFA, MTBSTFA, PFBBr, TFAA, TMPAH, TMSI

3 ANALYSIS

Inject 1 to 3 μ L of sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

THC	<i>d</i> ₃ -THC	Carboxy- Δ^9 -THC	<i>d</i> ₃ -Carboxy Δ^9 -THC
303**	306**	371**	374**
315	318	473	476
386	389	488	491

*Suggested internal standards for GC/MS: *d*₃-THC and *d*₃-Carboxy- Δ^9 -THC
Suggested internal standards for other GC: Cannabinol, Oxyphenbutazone, Ketoprofen

**Quantitation ion

ANALYTE NAME/MATRIX

M2741

Cannabinoids in Whole Blood

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER

**200 mg Bond Elut Certify II
12112080 or 12113051**

1 PRINCIPLE AND MECHANISMS

Acidic and neutral drug extraction using hydrophobic and anion-exchange mechanisms.

2 EXTRACTION METHOD

SAMPLE PREPARATION:

1. Add 0.8 mL 0.1 M K_2HPO_4 (pH adjusted to 7.0), to 1.0 mL blood
2. Add 200 μ L β -glucuronidase to the mixture, cap and vortex
3. Incubate at 37 °C for 16 to 18 hours. Add internal standards*
4. Add 4 mL acetonitrile dropwise while vortexing
5. Centrifuge; transfer supernatant to clean test tube
6. Add 16 mL 0.1 M K_2HPO_4 , pH 7.0/ CH_3OH (95:5) (v/v)

COLUMN PREPARATION/EXTRACTION:

1. Bond Elut Certify II Cartridge Conditioning

- a) 2 mL CH_3OH
- b) 2 mL 0.1 M K_2HPO_4 , pH 7.0/ CH_3OH (95:5) (v/v)

NOTE: Use a low vacuum (≤ 3 inches Hg) or gravity flow to prevent drying of sorbent.

2. Sample Loading

Load at 1 to 2 mL/minute

3. Column Wash

- a) 1 mL 0.1 M K_2HPO_4 , pH 7.0/ CH_3OH (95:5) (v/v); draw through under vacuum
- b) Dry for 10 seconds; stop vacuum immediately
- c) Add 100 μ L acetone; draw through under vacuum
- d) Dry for 2 minutes under vacuum.

NOTE: Do not exceed 2 minute drying time.

4. Elution I - THC

Elute 2 x 2 mL hexane/ethyl acetate (95:5) (v/v); collect eluate at 1 to 2 mL/minute.

NOTE: If hexahydrocannabinol as internal standard is being used, it will also elute in this fraction.

5. Column Wash

- a) Remove collection tubes
- b) Apply 5 mL 50% CH_3OH ; draw through under vacuum
- c) Apply 100 μ L ethyl acetate; draw through under vacuum
- d) Dry for 2 minutes under vacuum

NOTE: Do not exceed 2 minute drying time.

6. Elution II – THC Metabolite (THC-COOH)

Use either above collection tubes (if co-derivatization is to be performed) or clean ones for separate fractions. Elute 2 x 2 mL hexane/ethyl acetate (95:5) (v/v) with 1% acetic acid; collect eluate at 1 to 2 mL/minute.

DERIVATIZATION:

1. THC Alone

Add 50 μ L chloroform and 50 μ L TFA; vortex and cap. Heat at 70 °C for 10 minutes. Cool to ambient temperature. Evaporate to dryness. Reconstitute in 30 μ L heptane.

2. Combined Eluates or THC-COOH Alone

Add 50 μ L acetonitrile and 50 μ L BSTFA (with 1% TMCS). Blanket with N_2 and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA.

Other acceptable derivatization reagents: BSA, MSTFA, MTBSTFA, PFBBr, TFAA, TMPAH, TMSI

3 ANALYSIS

Inject 1 to 3 μ L of sample into chromatograph. Monitor the following ions for BSTFA derivatization (Mass Selective Detection):

THC	d_3 -THC	Carboxy- Δ^9 -THC	d_3 -Carboxy- Δ^9 -THC
303**	306**	371**	374**
315	318	473	476
386	389	488	491

*Suggested internal standards for GC/MS: d_3 -THC and d_3 -Carboxy- Δ^9 -THC
Suggested internal standards for other GC: Cannabinol, Hexahydrocannabinol, Oxyphenbutazone, Ketoprofen

**Quantitation ion

For more information, visit agilent.com/chem/Certify

APPENDIX



APPENDIX A: TREATMENT OF SERUM, PLASMA, OR WHOLE BLOOD SAMPLES

The following methods can be used to prepare serum, plasma, or whole blood samples, and are used to disrupt protein binding to drugs.*

SECTION 1

pH ADJUSTMENT

- A. In extreme pH values, such as greater than 9 or less than 3, buffer strengths greater than or equal to 0.1 M should be used.
- B. pH adjustment in stages (i.e. first buffering to pH 5, then to pH 3).
- C. A pH meter is a valuable tool for pH adjustments. Select the right meter for your needs from the range available from Agilent.

SECTION 2

PRECIPITATION

Using a polar solvent, such as acetonitrile, methanol, or acetone (generally 2 parts solvent per part of biological fluid), mix/vortex, then centrifuge down the precipitate, and remove the supernatant, which contains the drug.

- A. Drawback: the drug may be in part trapped in the precipitate.
- B. The organic solvent should be diluted with aqueous buffer to reduce the solvent strength and also to insure that the compounds of interest are in the proper ionization state.
- C. Acetonitrile is generally considered to be the most effective solvent for disrupting protein binding.
- D. Lower ratios of organic solvent to biological fluid may also be effective (i.e. 10-30% acetonitrile in plasma).

SECTION 3

ACID TREATMENT

Biological fluids can be treated with formic acid, perchloric acid, or trichloroacetic acid (i.e. 50 μ L of 0.1 M perchloric acid per 500 μ L plasma, or a 1:1 dilution of the biological fluid with 10% trichloroacetic acid).

SECTION 4

INORGANIC SALT TREATMENT

Biological fluids can be treated with salts such as ammonium sulfate or zinc sulfate to precipitate proteins.

SECTION 5

SONICATION

Sonicate the biological fluid for 15 minutes at room temperature, add an appropriate buffer (as described in the "Specimen Preparation" section of the extraction procedure for the particular drug), vortex 30 seconds, centrifuge at 2000 rpm for 15 minutes, and discard pellet.

*After treating the biological sample, the sample volume used in the drug extraction procedure should be taken from the treated sample.

SECTION 1

PURPOSE OF DERIVATIZATION

Derivatization of drugs prior to GC injection is important for any of three reasons. First, volatility or stability can be improved. Drugs with functional groups such as -COOH, -OH, -NH₂, and -NH tend to form intermolecular hydrogen bonds, decreasing their volatility. Second, derivatization can minimize interactions between the drugs and the column, which may interfere with the analysis. Active hydrogens interact destructively with the column's stationary phase, affecting both reproducibility and peak shape. Derivatization can minimize these interactions as well as improve peak resolution as coeluting compounds are separated. Finally, by increasing the bulk of the compound or by introducing atoms or functional groups that interact strongly with the detector, derivatization can also improve detectability.

SECTION 2

TYPES OF DERIVATIZATION

Silyl

There are three general types of reactions used to derivatize drug samples for GC analysis: silylation, alkylation, and acylation. Silyl derivatives are probably the most widely encountered for GC applications. They are usually formed by replacement of the active hydrogens with -SiR₃ groups. Most trimethylsilyl and t-butyldimethylsilyl derivatives have excellent thermal stability and are amenable to a wide range of injection and column conditions. Silylation reagents and the derivatized compounds are hydrolytically unstable, however, and must be protected from moisture.

Alkyl

Alkyl derivatization replaces active hydrogens with aliphatic or aromatic alkyl groups. Probably the largest application of alkylation for analytical derivatization is the conversion of organic acids into esters, especially methyl esters. The advantage of alkylation in this case is the excellent stability afforded by alkyl derivatives. They can be isolated and stored for extended periods if necessary.

Acyl

In acylation, drugs containing active hydrogens (such as in -OH and -NH groups) are converted to ester and amide derivatives. This type of derivative often produces a greater response to the chromatographic detection system than the parent compound in some ECD, TCD, and FID applications. In MS, acyl derivatives tend to direct the fragmentation patterns of compounds, which can provide useful structural information.

SECTION 3

COMMON DERIVATIZATION REAGENTS

Derivatization reactions should be quantitative, proceed rapidly, and produce products with the desired properties. As a result, many reagents are in common use and the type of derivatization employed will depend on the drug and the method of detection. Typical derivatization reagents and their abbreviations are shown in Table 1. Derivatization reagents suitable for specific drugs are listed in Table 2

Table 1. Common Derivatization Reagents

Abbreviation	Reagent	Type
BSA	<i>N,O</i> -bis(trimethylsilyl)acetamide	silyl
BSTFA	<i>N,O</i> -bis(trimethylsilyl)trifluoroacetamide	silyl
DMF-DMA	<i>N,N</i> -dimethylformamide dimethylacetal	alkyl
HFBA, HFBA, HFAA	heptafluorobutyric acid anhydride	acyl
MBTFA	<i>N</i> -methyl-bis(trifluoroacetamide)	acyl
MSTFA	<i>N</i> -methyl- <i>N</i> -trimethylsilyltrifluoroacetamide	silyl
MTBSTFA	<i>N</i> -methyl- <i>N</i> -(<i>t</i> -butyldimethylsilyl)trifluoroacetamide	silyl
PFBBr	pentafluorobenzyl bromide	alkyl
PFPA, PFPA, PFAA	pentafluoropropionic acid anhydride	acyl
TFAA	trifluoroacetic acid anhydride	acyl
TFAI	trifluoroacetylimidazole	acyl
TMPAH	trimethylanilinium hydroxide	alkyl
TMSC	trimethylsilyl chloride	silyl
TMSI	<i>N</i> -trimethylsilylimidazole	silyl

Table 2. Suitable Derivatization Reagents for Drugs of Abuse

Drug	Derivatization Reagent(s)	Drug	Derivatization Reagent(s)
amphetamine	BSTFA HFBA MSTFA TFAA	THC metabolites	BSA
			BSTFA
			MSTFA
			MTBSTFA
			PFBBr
			PFPA/HFIOH
			PFPA/PFPOH
			TFAA
methamphetamine	TFAA	LSD	TMPAH
			TMSI
			BSA
			BSTFA
barbiturates	BSTFA TMPAH DMF-DMA PFBBr	opiates	MSTFA
			TFAI
			BSTFA
			MBTFA
			PFPA
benzoyllecgonine	BSTFA PFPA	PCP	TFAA
			BSTFA
			HFBA

APPENDIX C: REFERENCES

Chen, Xiao-Hua. *Mixed-Mode Solid-Phase Extraction for the Screening of Drugs in Systematic Toxicological Analysis*. State University of Groningen: Ph.D. Dissertation. 1993.

Hawks, R. L.; Chiang, C. N., eds. *NIDA Research Monograph 73: Urine Testing for Drugs of Abuse*. Maryland: US Dept. of Health and Human Services. 1986.

McBay, A. J. in *Analytical Aspects of Drug Testing*, Deutsch, D. G., ed. New York: Wiley. 1989.

Pierce Catalog and Handbook. Rockford, IL: Pierce Chemical Company. 1994.

Sadee, W.; Beelen, G. C. M. *Drug Level Monitoring: Analytical Techniques, Metabolism, and Pharmacokinetics*. New York: Wiley. 1980.

Simpson, N.; Van Horne, K. C. *Sorbant Extraction Technology*. Harbor City, CA: Varian Sample Preparation Products. 1993.

United Nations International Drug Control Program. *Recommended Methods for the Detection and Assay of Heroin and Cannabinoids in Biological Specimens*. New York: United Nations. 1993.

United Nations International Drug Control Program. *Recommended Methods for the Detection and Assay of Cocaine, Amphetamine, Methamphetamine, and Ring-Substituted Amphetamine Derivatives in Biological Specimens*. New York: United Nations. 1993.

For additional information about drugs of abuse regulation and research, visit:

National Institute of Drug Abuse (NIDA) drugabuse.gov

Substance Abuse and Mental Health Services Administration (SAMHSA) samhsa.gov



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